The Technological and Nutritional Challenges of Producing Gluten-Free Pasta from Cassava and Banana Flours

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Adetiya Rachman

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Declaration

Some aspects of this thesis have been published or submitted in *peer-review* journals, and presented at conferences.

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Abstract of a thesis submitted in partial fulfilment of the requirements for the Degree of Doctor of Philosophy

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by

Adetiya Rachman

Pasta is a common cereal product made from wheat, and has affects the health of gluten intolerant people. A gluten-free diet is a must for coeliac patients and has recently become an option for a healthy lifestyle. Gluten-free materials have also become alternative sources in food products development in the wider food industry to counter the dependence on imported wheat. Some of the potential alternative base materials are banana and cassava flour due to their wide availability and physicochemical characteristics (Kumar, Saravanan, Sheeba, and Uma, 2019; Odey and Lee, 2020). However, there remains a challenge in developing good quality food products based on gluten-free materials since the absence of gluten can lead to technological limitations in forming good quality of food structures. The effort to improve the quality of cereal food products, alongside a healthy lifestyle choice, can be achieved by adding natural protein resources into the gluten-free flour mixture. Egg white protein has been proven to enhance gluten-free cereal products quality, as too has soy protein isolate, used as a vegetable protein source (Gao et al., 2018).

This study evaluated two different types of gluten-free flours, banana flour and cassava flour, that were combined in various formulations (0 to 100% proportion at 25% intervals) were used

to produce pasta. Subsequently two different protein additions (soy protein isolate and egg white protein) at different levels (0, 5, 10 and 15%) were used to stabilise pasta quality. The chemical, physical, textural, digestibility properties, nutritional, and sensory characteristics of the raw materials and cooked pasta were determined and compared to semolina pasta as a control. The research aimed to investigate the utilisation of alternative base materials (cassava and banana) and a technical additive (protein for gluten functionality and fortification) on the physical and nutritional quality of gluten-free pasta.

The assessment of the raw materials showed that banana and cassava flour had a lower protein content (4.54 and 1.41%) than semolina flour, however the amylopectin content and total dietary fibre contents were higher in banana and cassava flour (16.46 and 10.99%) compared to semolina flour (12.36 and 7.07%, respectively). Banana flour exhibited the highest resistant starch, total phenolic content (TPC), and antioxidant capacities, while cassava flour had the lowest values for these characteristics. The amino acid evaluation showed banana and cassava flour had a better ratio of total essential amino acid and total amino acid (35.37 and 29.95% versus 23.34%) but had lower limiting essential amino acid values (0.98 and 1.51% versus 11.12%) than semolina flour.

Gluten-free pasta producing from banana-cassava flour combinations resulted in higher dietary fibre content, lower protein content, and a darker colour compared to the semolina pasta control. All gluten-free pasta had lower optimum cooking time (2.8-4.2 min) and water absorption index (23.28-86.81%), but higher cooking loss (15.18-28.75%) than the control. Gluten-free pasta made from 75:25 banana : cassava flours and 100% banana flour had the best pasta-quality and the lowest reduced predictive glycaemic loading among other gluten-free pasta formulations. These formulations were selected for the following stage of this study.

The addition of soy protein isolate/egg white protein showed significant differences in physicochemical properties compared to semolina pasta, and gluten-free pasta with no protein enrichment. The level of protein addition increased the protein content of all gluten-free pasta formulations. Soy protein isolate addition gave higher protein content than egg white protein. There were no significant effects of both protein addition to either insoluble or soluble dietary fibre content. The protein fortification reduced a resistant starch content due to a lower portion of total starch in the gluten-free pasta composition. Cooking properties of pasta (optimum cooking time, swelling index, water absorption index, and cooking loss), and texture properties (firmness and extensibility), were affected by the level and the type of protein addition. Gluten-free pasta made from 75:25 banana : cassava flours showed similar trend to the gluten-free pasta made from 100% banana flour in physico-chemical, cooking properties and texture properties.

Egg white protein and soy protein isolate addition, and different gluten-free flour formulations, exhibited different effects on the nutritional and digestibility of gluten-free pasta. The addition of both proteins decreased starch digestibility, increased protein digestibility, improved amino acid profile, and protein digestibility-corrected amino acid score whereas only soy protein isolate enhanced the TPC and antioxidant capacity of the gluten-free pasta. A 25% cassava flour inclusion into gluten-free pasta formulation reduced the TPC and antioxidant capacities of the pasta. Gluten-free banana-cassava pasta with added egg white powder had better customer acceptance and purchase intent compared to soy protein isolate inclusion.

To sum up, banana and cassava flours could be utilised in gluten-free pasta production with high fibre content and low starch digestibility properties. Soy protein isolate and egg white protein could be incorporated into gluten-free pasta based on banana and cassava flours to promote pasta quality and nutritional properties of alternative functional products. Gluten-free pasta made of 75% banana flour and 25% cassava flour with 5% egg white protein performed the best gluten-free pasta quality, nutritional properties and moderate sensory acceptance.

Keywords: Banana flour, cassava flour, gluten-free pasta, protein addition, pasta-quality, nutritional properties

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Chapter 1

Introduction

1.1 Background

Pasta is a common food around the world due to its simplicity of production, good taste, and nutritional value. Most pasta manufacturers use wheat flour as a base ingredient, which is a health issue, especially for those people with coeliac disease. Coeliac patients are highly intolerant of the gluten present in wheat and other cereals (barley, oat and rye) (Hager, Zannini, and Arendt, 2012). The only proven treatment for coeliac disease is a restrictive diet with gluten-free products (Jayawardana, Montoya, McNabb, and Boland, 2019; Larretxi et al., 2020). Furthermore, a gluten-free diet has become a lifestyle choice for some non-coeliac people over and above the increasing diagnosis of being coeliac (Marti et al., 2014; Trevisan, Pasini, and Simonato, 2019).

Another significant issue related to wheat-based food production is the availability of raw materials. The commonly used pasta base materials, wheat grain, as well as the other gluten containing cereals, are not produced in some regions, especially in tropical countries. The limitation of raw material supply has been a problem in the development of pasta and bakery products in some non-wheat producing countries. They must import wheat grain or wheat flour to support their pasta making or other wheat-based industries. The need to develop non-wheat flours is not only due to economic and environmental reasons, but it is also to provide widely available materials for pasta production and other gluten-free products (Fiorda, Júnior, Silva, Souto, and Grosmann, 2013b; Odey and Lee, 2020).

The production of food products from alternative gluten-free sources is challenging because the absence of gluten gives rise to technological difficulties in creating a suitable food matrix. Most gluten-free pasta prepared from a single ingredient has an inferior quality compared to semolina pasta. Some suggested efforts to improve gluten-free pasta include the application of additives, improver materials (hydrocolloids and emulsifiers), crosslinking enzymes, or material pre-treatment such as starch pre-gelatinisation and fermented flour (Duta, Culetu, and Sozer, 2019; Odey and Lee, 2020; Rosa-Sibakov et al., 2016).

Two of the gluten-free flours that have the potential to be utilised to develop gluten-free pasta due to their wide availability, physicochemical, and nutritional content are cassava and banana flours. Cassava and banana are sixth and eighth greatest in terms of world crop production, they reached 278 billion kg and 116 billion kg, respectively, in 2108 (FAO, 2020). Cassava is a cheap source of flour and starch and provides significant calories for over 500 million people around the world. It is the third most important crop in tropical regions after rice and corn (Odey and Lee, 2020). Banana, on the other hand, is the second most important fruit worldwide after citrus and has become a functional ingredient because of its high resistant starch content (Kumar et al., 2019). It also has been reported that banana flour has high total phenolic content and antioxidant capacities (Bhatt and Patel, 2015; Darsini, Maheshu, Vishnupriya, and Sasikumar, 2012).

There are some studies that have proved that cassava and banana can be successfully utilised in pasta making as an additional ingredient or even as the base material. Various efforts to improve the quality of gluten-free pasta based on these materials have been made by the pre-treatment of the raw materials, modifying the production technique and the use of functional ingredients (Baah, Oduro, and Ellis, 2005; Fiorda et al., 2013b; Leonel, Souza, and Mischan, 2011; Odey and Lee, 2020; Radoi et al., 2015; Sarawong, Rodríguez Gutiérrez, Berghofer, and Schoenlechner, 2014a; Tiboonbun, Sungsri-in, and Moongngarm, 2011).

One of the challenges the food industry faces regarding the healthy lifestyle choices, is that of enhancing the nutritional values of gluten-free pasta by using natural protein sources such as egg powder and soy protein. It has been reported that the protein content of commercial gluten-free products were lower than regular wheat-based food products (Cornicelli, Saba, Machello, Silano, and Neuhold, 2018). Protein sources are preferable natural functional additives compared to hydrocolloid additives, with egg protein having been proved to improve the quality of gluten-free products, alongside soy protein isolate as the prefered option for egg-free products (Gao et al., 2018).

This study examined the use of banana and cassava flours as a single or composite raw material in producing gluten-free pasta. The physio-chemical, pasta quality, and glycaemic properties were investigated to determine the best proportion of banana and cassava flours in gluten-free pasta formulation. It was then followed by further study of incorporating egg white protein or soy protein isolate to gain the desirable pasta quality and nutritional properties and evaluate the consumer acceptance of gluten-free pasta based on banana and cassava flour. Specific characteristics such as cooking qualities, texture properties, protein, fibre, and resistant starch content, antioxidant properties, *in vitro* digestion properties, amino acid profiles, and sensory characteristics will be a benefit to illustrate the development of gluten-free pasta based on banana and cassava flour in comparison to conventional semolina pasta.

1.2 Aim of research

The aim of the research was to investigate the utilisation of alternative base materials (cassava and banana) and a technical additive (protein for gluten functionality and fortification) on the physical and nutritional quality of gluten-free pasta.

1.3 Objectives

The following were objectives to achieve the aim of the research:

1. To evaluate the effects of formulating of gluten-free pasta using alternative materials (cassava and banana) on the physical and glycaemic properties of gluten-free pasta.
2. To evaluate the effects of different types and levels of technical addition (egg, soy protein) on the physical quality of gluten-free pasta.
3. To investigate the nutritional properties of gluten-free pasta using *in vitro* starch digestion analysis, protein digestibility, amino acid profiles analysis, and sensory quality.

1.4 Hypothesis

The development of gluten-free pasta products has some limitations due to the absence of gluten and nutritional content of alternative base materials. There are at least three problems regarding the use of non-wheat materials in pasta making: finding a suitable gluten functionality replacement, improving the appropriate process, and maintaining the nutritional values. It has been hypothesized from the literature that:

1. Due to the low level of protein in cassava and banana, pasta made from these ingredients will have lower technical qualities compared with semolina pasta.

2. Durum pasta is technologically superior to gluten-free pasta; therefore, the addition of egg and soy protein will alter the pasta structure and improve its cooking properties compared with semolina pasta.
3. Fortification of gluten-free pasta will significantly affect the predictive glycaemic load due to protein starch interaction inhibiting enzymatic degradation.

1.5 Thesis structure

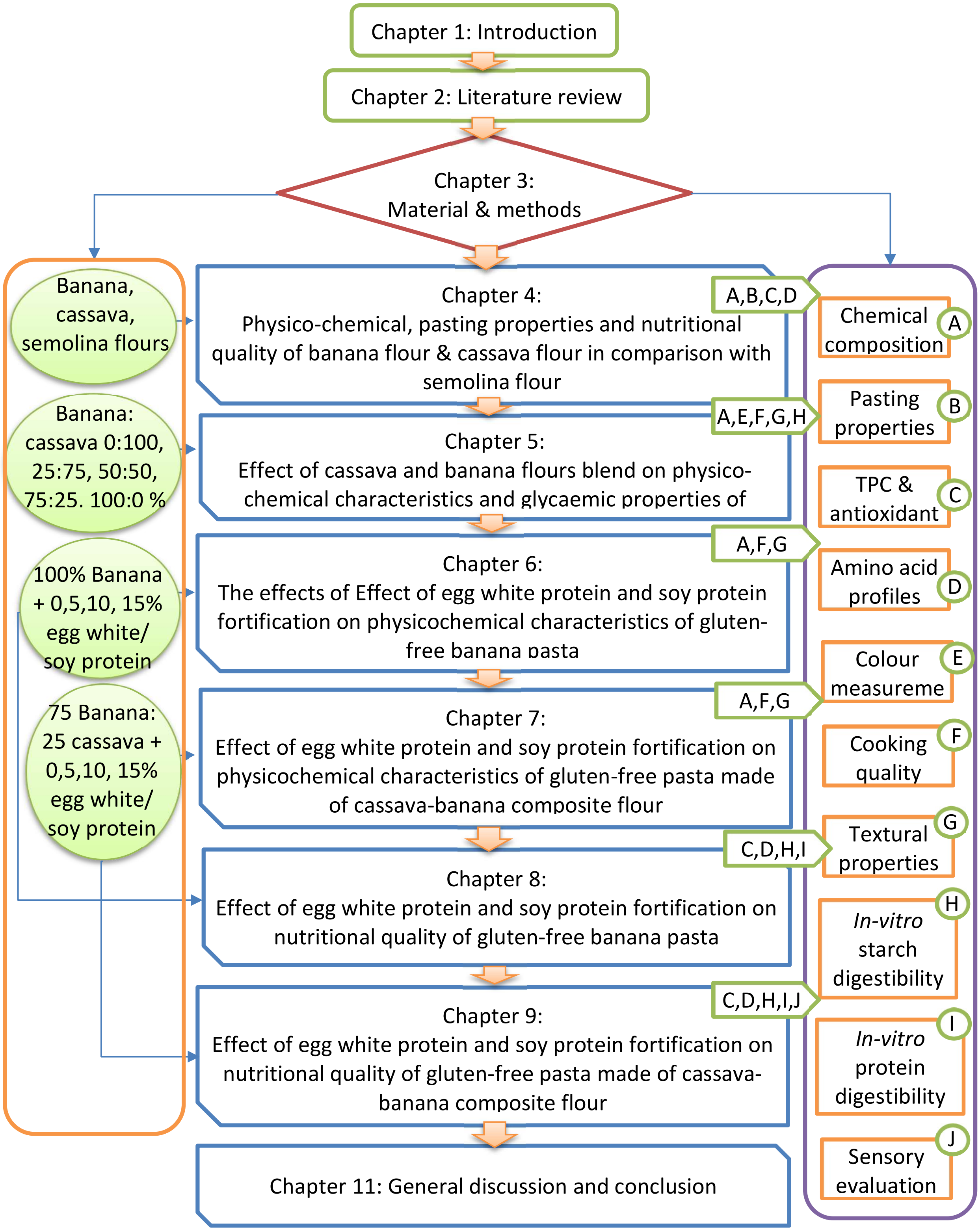

Figure 1.1 Thesis structure

Chapter 2

Literature Review

2.1 Pasta and gluten intolerance

2.1.1 Wheat-based pasta products

Pasta is traditionally produced from durum wheat flour as this flour has a high quality protein content that produces a desirable and well accepted pasta product. A high quality pasta exhibits low cooking loss, overcooking tolerance, high adhesiveness, high firmness, and no surface stickiness. The starch-protein interaction in durum wheat flour creates a firm matrix structure that is resistant to surface disintegration (Gao et al., 2018; Linares-García, Repo-Carrasco-Valencia, Glorio Paulet, and Schoenlechner, 2019; Morreale et al., 2019; Spinelli, Padalino, Costa, Del Nobile, and Conte, 2019).

The main protein content of wheat flour that responsible for creating the necessary characteristics of the dough is gluten. Gluten is a functional component part that gives viscoelasticity characteristics. In the presence of water and with mechanical processing, the starch and gluten components (gliadins and glutenin) create a binding complex called the gluten network. This network plays a basic role in the dough, it can retain fermentation gasses, it is easy to sheet and has a viscoelastic properties. These dough characteristics are needed in bakery manufacturing to create good products as well as in pasta making (Padalino, Conte, and Del Nobile, 2016; Woomer and Adedeji, 2020).

The development of alternative raw materials for pasta making leads to the utilisation of non-wheat flours and other non-gluten containing cereal, as a partial or whole substitution. This is mainly to provide alternative materials for gluten-free consumers and a wide variety of materials are available especially in developing countries (Odey and Lee, 2020; Woomer and Adedeji, 2020).

2.1.2 Gluten intolerance

The prevalence of gluten intolerance has increased significantly over the years. In total, around 0.3–0.6% of the world population, and up to 3%–10% in most wheat-consuming populations, suffer from gluten-related disorders (Jayawardana et al., 2019; Zevallos et al., 2017).

Gluten intolerance is mainly triggered by a gliadin component in gluten containing food. This protein component triggers an immunological process that is an abnormal condition for the intestine. Under this abnormal condition, the immune system in the intestine identifies gliadin as a threat component and creates a response which leads to digestive disorder (Proietti, Del Buono, Pagliaro, Del Buono, and Di Rienzo, 2013).

There are at least three diseases related to gluten intolerance, including coeliac disease, gluten sensitivity, and autism. Gluten sensitivity is similar to coeliac disease, which is a digestive disorder induced by gluten intake. These diseases are classified by the gluten reactivity in the intestine. While gluten sensitive patients only experience distress in their intestine, coeliac patients will suffer more widely because of gluten consumption. Gluten damages the intestine of coeliacs and causes it to malfunction thus it becomes unable to absorb nutrients (Proietti et al., 2013). This then leads to weight loss and other malnutrition disorders such as diarrhoea, anaemia, and fatigue (McAllister, Williams, and Clarke, 2019; Mohammadi, Azizi, Neyestani, Hosseini, and Mortazavian, 2015).

In the case of autism, it has been suggested, and it is still debated, that gluten consumption can cause behaviour disorders. Autism patients digest and absorb gluten and casein as abnormal peptide molecules which then influence the neurotransmission mechanism. These molecules disrupt the blood circulation to the brain and resulting in abnormal behaviour (Proietti et al., 2013).

Since these diseases are connected to genetic factors, there are no effective medical treatments to cure these diseases. The best treatment is to avoid consuming food containing gluten. Hence, a gluten-free diet is a must for these patients (Hager et al., 2012; Jayawardana et al., 2019; Mohammadi et al., 2015; Proietti et al., 2013).

For these reasons, there are numerous research projects to develop gluten-free pasta utilising gluten-free base material, technological processes, and technical addition. Some studies have focused on creating pasta with no gluten content, known as gluten-free pasta (Gao et al., 2018; Hager et al., 2012; Woomer and Adedeji, 2020).

2.2 Alternative base material for gluten-free pasta

There are various alternative gluten-free flours including legume, pseudo-cereals, and vegetable or fruits powders that have been used in gluten-free pasta production. Since these materials have no gluten content, the challenge is to develop viscoelastic characteristics and good texture of pasta. Hence, the main efforts are directed towards improving the quality, functionality, nutritional properties, and sensory quality of gluten-free pasta. This can be achieved by selecting appropriate raw materials and improving the technology process to meet standard quality and desirable pasta product (Gao et al., 2018; Padalino et al., 2016; Woomer and Adedeji, 2020).

As mentioned previously, cassava flour and banana flour can be suitable alternatives for producing gluten-free pasta not only for their widely availability, but also for their physico-chemical and nutritional properties. Cassava flour contains high amount of carbohydrate (85-92%), a small amount of fat and protein (0.92-0.96% and 0.44-1%, respectively), and some resistant starch and fibre (0.1-2.2% and 2-5%, respectively) (Odey and Lee, 2020; Oluba, Oredokun-Lache, and Odutuga, 2017; Pereira and Leonel, 2014). Compared to cassava flour, banana flour has a lower carbohydrate content (80-83%), higher protein content (3-5%), a variable fat content (0.1-3%), and much higher resistant starch and fibre content (31-46% and 7-15%, respectively) (Campuzano, Rosell, and Cornejo, 2018; da Mota, Lajolo, Cordenunsi, and Ciacco, 2000; Kumar et al., 2019). Banana flour has also been found to have a high total phenolic content (44-145 mg/100 g) and antioxidant activities (0.7-1 mg/g of DPPH, 2-7 µmol/mg of ferric reducing/antioxidant power (FRAP) values) and low glycaemic properties that made it a healthy food source (Kumar et al., 2019).

2.2.1 Banana flour utilisation in gluten-free pasta production

There are numerous studies that have utilised banana flour in producing wheat-based pasta or gluten-free pasta (Table 2.1). However, there are far less studies that have used banana flour as a single base material (Cheok et al., 2018; Zandonadi et al., 2012).

Table 2.1 Banana flour utilisation in pasta production

Materials	Technology & additives	Reference
Wheat-based		
Semolina flour	15-35% unripe plantain flour	Garcia-Valle, Agama-Acevedo, Alvarez-Ramirez, and Bello-Perez (2019)
Wheat flour	15-30% unripe banana pulp/peel	Castelo-Branco et al. (2017)
Wheat flour	30% banana flour, β-glucan	Choo and Aziz (2010)
Semolina flour	15-45% unripe banana flour	Agama-Acevedo et al. (2009b)
Gluten-free		
Maize flour (73.5%) chickpea flour (12.5%), unripe plantain flour (12.5%)	Carboxy methyl cellulose (1.5%)	Agama-Acevedo, Bello-Perez, Pacheco-Vargas, Tovar, and Sáyago-Ayerdi (2019)
Maize flour (75%) chickpea flour (12.5%), pulp/whole unripe plantain flour (12.5%)	Carboxy methyl cellulose (1.5%)	Patiño-Rodríguez, Bello-Pérez, Flores-Silva, Sánchez-Rivera, and Romero-Bastida (2018)
Green banana flour (47%)	Egg white (31.5%), guar gum (2.5%), xanthan gum (2.5%)	Cheok et al. (2018)
Rice flour (60-100%), banana powder / fresh banana / fresh banana with ascorbic acid pre-treatment (0-40%)	1 fresh whole egg per 100 g flour	Radoi et al. (2015)
Chickpea flour (60-70%), unripe plantain flour (15-30%), maize flour (0-20%)	Carboxy methyl cellulose (0.5%)	Flores-Silva, Berrios, Pan, Osorio-Díaz, and Bello-Pérez (2014)
Rice flour (40-85%), green plantain flour (15-60%)	Distilled monoglyceride (0.5%), pre-gelatinisation flour, egg albumen (3.5-6%)	Sarawong et al. (2014a)
Green banana flour (47%)	Egg white (31.5%), guar gum (2.5%), xanthan gum (2.5%)	Zandonadi et al. (2012)
Rice flour (0-80%), green banana flour (0-80%), tapioca flour (20%)	Noodle processing with steaming and shaping	Tiboonbun et al. (2011)

The use of 30% green banana flour to replace rice flour gave the best gluten-free pasta quality among other formulations (Sarawong et al., 2014a). This research only used 6% egg albumen, but it implemented pre-treatment on rice flour (pregelatinized rice flour). Nutritional values were not analysed in the research. The use of 12.5% unripe banana flour has also been studied in gluten-free pasta formulation based on maize (73.5-75%) and chickpea flour (12.5%) which showed a significant increase in dietary fibre and had medium glycaemic index (66) (Agama-Acevedo et al., 2019; Patiño-Rodríguez et al., 2018).

Gluten-free pasta made of unripe banana flour as a single raw material had a greater sensory quality compared with wheat-based pasta. Different types of banana cultivar have also been reported to give significant sensory acceptance (Cheok et al., 2018; Zandonadi et al., 2012). The disadvantage found in these studies was the high amount of egg white composition (31.5%) required to make an acceptable product compared to similar studies in gluten-free pasta based on banana flour that applied up to 6% egg compotition (Radoi et al., 2015; Sarawong et al., 2014a) . However, it used less egg portion compared to the wheat pasta control (39.4%), therefore protein content was much lower (9.30% of 19.3%). It also used hydrocolloids as the additive to improve pasta quality (Cheok et al., 2018; Zandonadi et al., 2012).

2.2.2 Cassava flour utilisation in gluten-free pasta production

Cassava flour utilisation either in wheat-based or gluten-free pasta is still limited. Most research has implemented a pre-gelatinisation process of cassava flour to produce desirable pasta, while some of them used functional additives to improve pasta quality as can be seen in Table 2.2.

Table 2.2 Cassava flour utilisation in pasta production

Base materials	Technology & additives	Reference
Wheat-based		
Wheat flour	Fermented cassava flour (50%) replacement with pre-gelatinisation	Odey and Lee (2020)
Durum wheat semolina	Cassava flour replacement (0-50%), Arabic gum (2%), gelatine (3%), sodium carbonate (0.1%) and potassium carbonate (0.1%)	Oladunmoye, Aworh, Ade-Omowaye, and Elemo (2017)
Wheat flour	Cassava flour (0-50%) replacement with pre-gelatinisation	Baah et al. (2005)
Wheat flour	Cassava flour (30%) and cassava mucilage (0-1%)	Charles et al. (2007)
Gluten-free		
Cassava flour (60-70%), amaranth flour (30-40%)	Carboxy methyl cellulose (0.21-0.25%), egg powder (2%), enzyme Veron (0.03%)	Ramirez et al. (2019)
Cassava starch (60%), cassava flour (10%), amaranth flour (30%)	Pre-gelatinisation flour, fresh whole egg (48 % w/w dry mixture)	Fiorda, Junior, da Silva, Souto, and Grosmann (2013a)

Fiorda et al. (2013a) succeeded in using 60% cassava starch with 10% pregelatinized cassava flour and 30% amaranth flour to give the best quality with good consumer acceptance (7.2 out of 9 scale) of a gluten-free pasta product. Their research found that the amount of cassava flour and starch in gluten-free pasta formulation altered cooking properties (decreased optimum cooking time, decreased mass increase and increased loss of solids). Ramirez et al. (2019) observed a lower hardness with increased cassava flour portion in gluten-free pasta formulation which showed the effect of cassava flour on the textural properties of gluten-free pasta.

2.3 Technological challenges in producing gluten-free pasta

Most of the research to date has focused on the finding of appropriate materials and additives; there is scant research on the role of the processing method (Hager et al., 2012). Some research projects avoid using the additives for gluten functionality replacement and prefer to modify the process. The process can be improved by the pre-treatment of ingredients or by using a higher temperature in the extrusion process (Marti et al., 2014). Research on comparing conventional extrusion (50°C) with extrusion cooking (115°C) showed that advanced cooking improved the cooking quality of rice based pasta (cooking losses and stickiness) (Marti, Pagani, and Seetharaman, 2011; Marti, Seetharaman, and Pagani, 2010). It has also been suggested that the production of fortified pasta needs a modification of hydration level and mixing speed of extrusion. A higher hydration level and mixing speed should be applied to limit the agglomeration of particles during the production process (Duta et al., 2019).

The use of additives (hydrocolloids (gums or carboxyl methyl cellulose, for example) and emulsifiers) in gluten-free pasta lead to "artificial food" from the consumers' perspective (Marti et al., 2013; Morreale et al., 2019). Hence, it is not surprising that the most used technical addition in commercial gluten-free products is egg powder (Gao et al., 2018; Hager et al., 2012).

The challenge is to provide applicable technology that not only improves gluten-free pasta quality but also accommodates the need to keep the product as natural as possible which can be done by the addition of a protein source into the gluten-free pasta formulation. The most common protein sources utilised in producing gluten-free pasta are egg white protein, whey protein, soy protein, and fish powder. Egg white protein has been shown to improve the quality of pasta due to its viscoelasticity characteristics, while soy protein isolate has become the most popular plant protein that provides an alternative option for vegetarian or other dietary restrictions (Linares-García et al., 2019; Zhang et al., 2020).

2.3.1 Egg white protein application in gluten-free pasta products

Egg white protein has been used as a functional ingredient to replace gluten functionality in gluten-free pasta products. Table 2.3. shows that egg white protein can be applied to enhance the quality of gluten-free pasta, while some of the research papers added a combination of other additives in their formulation. Some of the research applied egg protein at a fixed proportion, while others used variable values to determine the optimum proportion that gave the best effect on the quality of gluten-free pasta. However, since there are many varieties of base material used, there is not any level of addition of egg white protein that is suggested in producing gluten-free pasta.

It is also noted that egg white protein was employed more frequently than egg yolk or whole egg. Egg white protein was shown to improve the cooking properties, while egg yolk improved the textural properties of gluten-free pasta (Palavecino et al., 2017). From a consumer perspective, it was found that egg white protein application in gluten-free pasta had a better sensory acceptance (above 7 out of 9) compared to egg yolk or whole egg (5 out of 9) (Witek, Maciejaszek, and Surówka, 2020).

Table 2.3 Egg protein utilisation in gluten-free pasta products

Based materials	Additives	Reference
Rice flour (67%)	Freeze-dried whole egg / egg white / yolk (0-6%)	Witek et al. (2020)
Chickpea flour (65.7%)	Egg white (34.5%)	de Lima, Botelho, and Zandonadi (2019)
Pregelatinized rice flour (60-90%), soybean flour (0-20%), orange-fleshed sweet potato (0-10%)	Liquid egg albumen (10% w/w dry material)	Marengo et al. (2018)
Sorghum flour (70-100%)	Xanthan gum (0-2.5%) / egg albumen (0-11%) / egg powder (0-9%) / pregelatinized corn starch (0-30%) (w/w dry material)	Palavecino et al. (2017)
Sorghum flour (40-60%), rice flour (15–30%), and/or corn (10–20%) and potato starch (10–40%)	Whole egg (25% w/w flour), soybean oil (2% v/w flour)	Ferreira et al. (2016)
corn starch and corn flour mixture (4:1) (49.6-56.2%)	Dried whole egg (2.45-6%), egg white (0.25-0.6%), mix of xanthan gum and locust bean gum (2:1) (2.5%)	Larrosa, Lorenzo, Zaritzky, and Califano (2016)
Parboiled milled rice (85-100%)	Egg white protein (0-15%) / Whey protein (0-5%)	Marti et al. (2014)
Corn starch (42.8%) and corn flour (10.7%)	Dried whole egg and egg white (0.7-6.7%), xanthan gum or locust bean gum (0.5-6.5%)	Larrosa, Lorenzo, Zaritzky, and Califano (2013)
Teff flour (62.8%) / Oat flour (64.7%)	Egg white powder (9.7-11%) and pasta emulsifier (1.1-1.3%)	Hager, Czerny, Bez, Zannini, and Arendt (2013)
Buckwheat (40%), rice (25-27%)	Fresh whole egg (30%)	Alamprese, Casiraghi, and Pagani (2007)

2.3.2 Soy protein application in gluten-free pasta products

Soy protein utilisation as an additive in cereal products is mainly because of its functionality and nutrition content. There are different types of soy protein derived from different processes, including full-fat soy flour (FFSF), defatted soy flour (DSF) soy protein concentrate, and soy protein isolate. FFSF is a common soy flour from soybean milling, while DSF is a by-product of oil extraction from FFSF. Soy protein concentrate and soy protein isolate come from different fractionation processes. Aqueous alcohol extraction produces soy protein concentrate, while extraction via alkali and acid precipitation produces soy protein isolate (Duque-Estrada, Kyriakopoulou, de Groot, van der Goot, and Berton-Carabin, 2020). Soy protein utilisation in gluten-free pasta is presented in Table 2.4.

The most frequently used soy protein in gluten-free development is soy protein isolate. Limroongreungrat and Huang (2007) found that type of soy protein (soy protein concentrate or DSF) did not give significant differences in cooking quality and texture properties of gluten-free pasta made of sweet potato flour. It was reported that 15% soy protein inclusion was considered as an optimum condition in improving gluten-free pasta quality (Limroongreungrat and Huang, 2007; Udachan and Sahoo, 2017).

Table 2.4 **Soy protein utilisation in gluten-free pasta products**

Base materials	Additives	Reference
Broken rice flour (75-100%)	Defatted soy flour (0-25%)	Udachan and Sahoo (2017)
Rice flour (57-67%)	Soy protein concentrate / egg albumen / rice bran protein concentrate / whey protein concentrate (0-9%), distilled monoglyceride (1%)	Phongthai, D'Amico, Schoenlechner, Homthawornchoo, and Rawdkuen (2017)
Rice flour (81-90%) Rice waxy flour (9-10%)	Soy protein isolate (0-10%)	Detchewa, Thongngam, Jane, and Naivikul (2016)
Rice flour (90-100%)	Soy protein isolate (10% w/w flour) / ogaja fruit extract (7 % w/w flour)	Lee et al. (2016)
Rice flour (70-100%)	Soy protein isolate (0-20%) / dehydrated egg albumen (0-15%) / pregelatinized rice flour (0-30%)	Schmiele, Jaekel, Ishida, Chang, and Steel (2013)
Maize flour (20-35%), quinoa (0-30%)	Defatted soy flour (2.5-10%)	Mastromatteo, Chillo, Iannetti, Civica, and Del Nobile (2011)
Amaranth flour (0-50%), quinoa flour (0-60%), buckwheat flour (0-100%)	Soy protein isolate / egg white powder / casein (0-18% w/w flour), emulsifier (1.2% w/w flour)	Schoenlechner, Drausinger, Ottenschlaeger, Jurackova, and Berghofer (2010)
Sweet potato flour (55-100 %)	Soy protein concentrate / defatted soy flour (0-45%)	Limroongreungrat and Huang (2007)

2.4 Nutritional challenges of gluten-free pasta product

There are several studies that provide nutritional and digestibility assessment to support the nutritional challenge in gluten-free pasta development. The nutritional evaluation of gluten-free pasta, containing banana flour or cassava flour or egg white protein or soy protein, is shown in Table 2.5.

Table 2.5 Nutrition assessment in gluten-free pasta products

Base materials	Additives	Nutritional aspects	Reference
Maize, chickpea, & **unripe plantain flour**	Carboxy methyl cellulose	Protein, fat, ash, total starch, dietary fibre, predicted glycaemic index (pGI)	Agama-Acevedo et al. (2019)
Maize, chickpea, & pulp/whole **unripe plantain flour**	Carboxy methyl cellulose	Protein, fat, ash, total starch, dietary fibre, metabolite profile	Patiño-Rodríguez et al. (2018)
Rice flour, **banana powder** / fresh banana / fresh banana with ascorbic acid pre-treatment	1 fresh whole egg per 100 g flour	Total phenolic content, cinnamic acid, microelements composition (Mn, Cu, Ni, Zn, Fe)	Radoi et al. (2015)
Chickpea, **unripe plantain**, & maize flour	Carboxy methyl cellulose	Total starch, resistant starch, starch digestibility & pGI	Flores-Silva et al. (2014)
Rice flour & **green plantain flour**	Distilled monoglyceride & pre-gelatinisation flour	Resistant starch	Sarawong et al. (2014a)
Green banana flour	**Egg white**, guar gum, & xanthan gum	Protein, fat, ash, total starch, dietary fibre	Zandonadi et al. (2012)
Cassava flour & amaranth flour	Carboxy methyl cellulose, **egg powder**, & enzyme Veron	Total starch, protein, & ash	Ramirez et al. (2019)
Rice flour	Freeze-dried whole egg / **egg white** / yolk	Total starch, protein, & ash	Witek et al. (2020)
Chickpea flour	**Egg white**	Protein, fat, total starch, dietary fibre	de Lima, Botelho, and Zandonadi (2019)
Sorghum flour, rice flour, and/or corn, and potato starch	**Whole egg**, soybean oil	Protein, fat, total starch, and ash	Ferreira et al. (2016)
Teff flour / Oat flour	**Egg white powder** and pasta emulsifier	Protein, fat, ash, total starch, dietary fibre, starch digestibility & pGI	Hager et al. (2013)
Broken rice flour	**Defatted soy flour**	Protein, fat, ash, total starch, crude fibre	Udachan and Sahoo (2017)
Sweet potato flour	**Soy protein concentrate / defatted soy flour**	Protein, β-carotene	Limroongreungrat and Huang (2007)

The nutritional studies of gluten-free pasta containing banana flour and/or egg white protein were more advanced compared to cassava flour and soy protein isolate (Table 2.4). The gluten-free pasta based on banana flour studies also focused on a resistant starch content as this flour contains a high amount of resistant starch and dietary fibre and led to higher amounts of resistant starch and dietary fibre in the gluten-free pasta (Flores-Silva et al., 2014; Sarawong et al., 2014a). Radoi et al. (2015) provided the total phenolic content and mineral compositions and found banana inclusion improved iron and manganese content as well as increasing the total phenolic content compared to rice pasta control. Agama-Acevedo et al. (2019) and Flores-Silva et al. (2014) analysed digestibility properties and found banana pasta altered glycaemic index and had lower values than conventional semolina pasta, but neither of them evaluated protein digestibility. Protein digestibility determines how much protein can be digested by enzymes which contribute to nutritional quality alongside the amino acid profiles (Rafiq, Sharma, and Singh, 2017).

The assessment of nutritional quality in gluten-free pasta containing cassava flour or soy protein isolate on the other hand was limited to proximate analysis (Protein, fat, ash, total starch, and dietary/crude fibre). This should be rectified since soy protein has highly beneficial nutritional properties being rich in antioxidant ability and providing some essential amino acids (Beatriz Cervejeira and Adelaide Del Pino, 2011; Corgneau et al., 2019). It also has been reported that soy protein isolate is used in a variety of food products because of its high protein digestibility (Djuardi, Yuliana, Ogawa, Akazawa, and Suhartono, 2020).

The challenge to improve the nutritional and digestibility properties of gluten-free pasta can be achieved by performing a comprehensive nutritional evaluation. That comprehensive nutritional evaluation will include not only proximate analysis but also total phenolic content, antioxidant properties, amino acid profiles, starch digestibility, and protein digestibility. These analyses will give detailed information not only of the comparable quality of the gluten-free will contribute to a better understanding of the nutritional properties compared to conventional semolina pasta.

Chapter 3
Material and Methods

3.1 Pasta ingredients

Green banana flour (*Musa cavendishii*), and cassava flour (*Manihot esculanta*), were purchased from Food Compass Limited, Auckland, New Zealand. Egg white protein powder (80% protein content) was obtained from Nothing Naughty Limited, Tauranga, New Zealand, and soy protein isolate (91% protein content) was obtained from Bulk Powder Limited, Braeside, Australia. Durum semolina (Sun Valley Foods Limited, Auckland, New Zealand) was used to produce a control pasta.

3.2 Methods

This research was divided into three stages of experiment;

(1) The evaluation of gluten-free pasta formulation using cassava and banana flours on physicochemical and glycaemic properties of gluten-free pasta (Chapter 4 and Chapter 5)

(2) The evaluation of the effects of different types and levels of egg white protein and soy protein isolate on the physical quality of gluten-free pasta (Chapter 6 and Chapter 7)

(3) The investigation of nutritional properties of gluten-free pasta using *in vitro* starch digestion analysis, protein digestibility, amino acid profiles analysis and sensory quality (Chapter 8 and Chapter 9).

3.2.1 Banana-cassava composite flour blend for pasta making

Banana flour and cassava flour were used as a single material or blended in different portions to make composite flours as follows:

Table 3.1 Composition of banana and cassava flours for composite flour used to prepare pasta per 100 g

Sample	Cassava flour (g)	Banana flour (g)	Water (ml)
100% Cassava	100	-	50
75% Cassava: 25% Banana	75	25	50
50% Cassava: 50% Banana	50	50	50
25% Cassava: 75% Banana	25	75	60
100% Banana flour	-	100	70

After the initial experiments to determine the ideal balance of cassava and banana, a second round of experiments was conducted to try to improve the pasta by fortifying with protein.

3.2.2 Protein fortified flour blend for gluten-free banana pasta making

Either egg white protein or soy protein isolate was added to banana flour as follows:

Table 3.2 Composition of banana flour with protein addition used to prepare pasta per 100 g

Sample	Banana flour (g)	Egg white protein (g)	Soy protein isolate (g)
Banana pasta	100	-	-
BE5	100	5	-
BS5	100	-	5
BE10	100	10	0
BS10	100	-	10
BE15	100	15	-
BS15	100	-	15

BE = Banana + egg protein. BS = Banana + soy protein

3.2.3 Protein fortified flour blend for gluten-free banana-cassava pasta making

Banana-cassava composite flour was fortified with either egg white protein or soy protein isolate with the formulation as follow:

Table 3.3 Composition of banana flour, cassava flour with protein addition used to prepare pasta per 100 g

Sample	Banana flour (g)	Cassava flour (g)	Egg white protein (g)	Soy protein isolate (g)
Banana-cassava pasta	75	25	-	-
BCE5	75	25	5	-
BCS5	75	25	-	5
BCE10	75	25	10	-
BCS10	75	25	-	10
BCE15	75	25	15	-
BCS15	75	25	-	15

BCE = Banana-cassava flour + egg protein. BCS = Banana-cassava flour + soy protein

3.2.4 Pasta preparation for banana-cassava composite flour blend

Pasta was produced using a pasta making machine fitted with a spaghetti die (2.25 mm diameter of die hole; model: MPF15N235M, manufactured by Fimar, Villa Verucchio, Ravenna, Italy). Samples were mixed in the pasta maker for 4 min to ensure a uniform mixture of flour components. Pasta batches (300 g) were mixed with an appropriate quantity of tap water (41°C) for 20 min. The control pasta used 30% tap water based on previous findings (Foschia, Peressini, Sensidoni, Brennan, and Brennan, 2014). Initial experiments were conducted to determine the optimum quantity of water as shown in Table 3.1. Boiling water (100°C) was used for gluten-free pasta making as this led to better extrusion results. The pasta was extruded using 20 holes spaghetti die of 2.25 mm diameter. Samples were then cooked on the same day and tested for textural properties test, the rest of the samples were put in sealed bags and stored at-18°C. All pasta was defrosted for 10 min at room temperature prior to analysis.

3.2.5 Pasta preparation for protein fortified flour blend

The pasta was produced using a pasta making machine (model: MPF15N235M, Fimar, Ravenna, Italy) fitted with a spaghetti die (2.25 mm diameter). All the dry materials were mixed in the pasta maker for 4 min to ensure a uniform mixing (300 g). The blends of gluten-free formulation then were mixed with up to 70% (w/v) boiling water (100°C) for 20 min. The control pasta was made from semolina flour with 40% (w/v) water (42°C). The pasta was then extruded, collected on a plastic tray, and cut into lengths of approximately 25 cm.

3.2.6 Moisture content

Moisture content of pasta and raw materials was determined using the standard American Association of Cereal Chemists method (AACC, 2002). Aluminium pans were labelled and weighed using an analytical balance (ARC120; OHAUS Corp., Parsippany, NJ, USA). Samples (5 g) were ground and placed into aluminium pan then put in the oven at 105°C overnight. Dried sample in the pan was cooled to room temperature in a desiccator for 1 h before reweighing. Moisture content was counted based on following equation (AACC, 2002):

$$\text{Moisture content (\%)} = \frac{\text{Loss of weight}}{\text{Sample weight}} \times 100$$

3.2.7 Total starch content

Total starch content was measured using the Megazyme total starch assay procedure K-TSTA-50A/K-TSTA-100A 06/17 (Megazyme Ltd., Wicklow, Ireland) based on AOAC official method 996.11 (AOAC, 1996). A ground sample (100 mg) was placed into glass tube (16 x 120 mm) and added 0.2 mL of aqueous ethanol (80% v/v). The tube was mixed on a vortex mixer and a magnetic stirrer bar (5 x 15 mm) was placed in it. Two mL of 2 M KOH was added to each tube and the pellets were resuspended by stirring for approximately 20 min in an ice/water bath over a magnetic stirrer. Sodium acetate buffer (pH 3.8) was added to each tube with stirring followed by 0.1 mL of thermostable a-amylase and 0.1 mL of amyloglucosidase. Tubes were mixed well and placed in a water bath at 50°C for 30 min with intermittent vortex mixing. The contents of the tube were quantitatively transferred to a 100 mL volumetric flask and made to volume using distilled water then mixed well. An aliquot (approximately 15 mL) was transferred to 15 mL Falcon tube and centrifuged at 1800 g for 10 min. Glass tubes (16 x 100 mm) were prepared in a rack and filled with a centrifuged aliquot (0.1 mL) alongside with D-glucose controls (0.1 mL) and blanks (0.1 mL distilled water). GOPOD reagent (3.0 mL) was added to each tube and the tubes were incubated at 50°C for 20 min. The absorbance of each sample and the D-glucose control were measured at 510 nm against the reagent blank. Total starch (%) was calculated as (AOAC, 1996):

$$\text{Total starch } = \Delta \text{A} \times \frac{\text{F}}{\text{W}} \times 90$$

Where:

ΔA	=	Sample absorbance
F	=	100 (µg of D-glucose) divided by the D-glucose standard absorbance
W	=	Sample weight (mg)

3.2.8 Amylose-amylopectin content

Amylose-amylopectin content was determined using the Megazyme amylose/amylopectin assay procedure K-AMYL 06/18 (Megazyme Ltd., Wicklow, Ireland) (Yun and Matheson, 1990). A flour sample of 20 mg was placed into a 10 mL screw capped Kimax® sample tube, added 1 mL of DMSO (dimethyl sulphoxide) and heated in a boiling water bath for 1 min. The sealed tube was mixed at high speed on a vortex mixer and placed in a boiling water bath for 15 min, with intermittent high speed mixing on a vortex mixer. The tube was allowed to stand at room temperature for 5 min and then 2 mL of 95% (v/v) ethanol was added with continuous mixing on a vortex mixer. A further 4 mL ethanol was added, and the tube was allowed to stand at room temperature for 15 min. The tubes were centrifuged at 2000 g for 5 min and the supernatant was discarded. The starch pellet was resuspended in 2 mL of DMSO. The tube was placed in a boiling water bath for 15 min and mixed occasionally. Con A solvent (4 mL) was added and the tube contents were transferred to a 25 mL volumetric flask and diluted with Con A solvent. This was labelled as a solution A.

A 1 mL aliquot of solution A was transferred to a 2.0 mL Eppendorf® microfuge tube and 0.50 mL of Con A solution was added. The tube was stored for 1 h at room temperature before being centrifuged at 14000 g for 10 min in at room temperature. The supernatant (1 mL) was transferred to a 15 mL centrifuge tube. Then 3 mL of 100 mM sodium acetate buffer, pH 4.5, was added and the tube was heated in a boiling water bath for 5 min before being placed in a water bath at 40°C for 5 min. Amyloglucosidase/α-amylase enzyme mixture (0.1 mL) was added to the tube and it was incubated at 40°C for 30 min. The tube was centrifuged at 2000 g for 5 min. Aliquots (1.0 mL) of the supernatant were placed into glass tubes, with 4 mL of GOPOD reagent and then incubated at 40°C for 20 min, together with a reagent blank and the

D-glucose controls. The absorbance of each sample and the D-glucose controls were read at 510 nm against the reagent blank. This was the Con A supernatant absorbance.

Total starch absorbance was determined by mixing 0.5 mL of solution A with 4 mL of 100 mM sodium acetate buffer, pH 4.5. To this mixture 0.1 mL of amyloglucosidase/α-amylase solution was added before being incubated at 40°C for 10 min. An aliquot (1.0 mL) and the D-glucose standard were placed into glass test tubes, with 4 mL of GOPOD reagent and incubated at 40°C for 20 min. The absorbance of each sample the D-glucose controls were read at 510 nm against the reagent blank. This value determined total starch absorbance. Amylose content (%) was calculated as follow (Yun and Matheson, 1990):

$$\text{Amylose content } (\%) = \frac{\text{Con A supernatant absorbance}}{\text{Total starch absorbance}} \times 66.8$$

3.2.9 Resistant starch content

Resistant starch (RS) was determined using the Megazyme resistant starch assay procedure K-RSTAR 06/18 (Megazyme Ltd., Wicklow, Ireland) based on the modified method described by AOAC (2002). A ground sample (100 $\pm$ 5 mg) was placed into screw cap tube (Corning® culture tube; 16 x 125 mm) and added 4.0 mL of pancreatic α-amylase (10 mg/mL) containing AMG (3 U/mL) (Solution 2). The tube was mixed with a vortex mixer and incubated in a shaking water bath at 37°C with continuous shaking (200 strokes /min) for 16 h.

The tube was removed from the water bath and 4.0 mL of ethanol (99% v/v) was added. The capped tube then was centrifuged at 1500 g for 10 min. The supernatant was removed, and 2 mL of 50% ethanol was used to resuspend the pellet and was then mixed with a vortex mixer. A further 6 mL of 50% ethanol was added, mixed, and centrifuged again at 1500 g for 10 min.

The supernatant was removed, and this suspension and centrifugation step was repeated once more. The supernatant was removed, and the tube was inverted to drain all excess liquid.

A magnetic stirrer bar (5 x 15 mm) and 2 mL of 2 M KOH was added to each tube and the tube was stirred for 20 min in an ice/water bath over a magnetic stirrer. Then 8 mL of 1.2 M sodium acetate buffer (pH 3.8) was added while stirring on a magnetic stirrer followed by 0.1 mL of amyloglucosidase, they were mixed well and incubated in a water bath at 50°C for 30 min with intermittent mixing on a vortex mixer. The contents of the tube quantitatively transferred to a 100 mL volumetric flask and adjusted to 100 mL with distilled water. An aliquot (approx. 15 mL) was placed in Falcon plastic tube and centrifuged at 1500 g for 10 min. The supernatant (0.1 mL), blank (0.1 mL distilled water), and D-glucose standard (0.1 mL) were placed into glass test tubes (16 x 100 mm), 3.0 mL of GOPOD reagent was added and it was incubated at 50°C for 20 min. The absorbance of each solution was measured at 510 nm against the reagent blank. Resistant starch (%) was determined with the following equation (AOAC, 2002):

$$\text{Resistant starch} = \Delta \text{E} \times \frac{\text{F}}{\text{W}} \times 90$$

Where:
ΔE = Sample absorbance
F = 100 (µg of D-glucose) divided by the D-glucose standard absorbance
W = Sample weight (mg)

3.2.10 Protein content

The protein content was determined using the Dumas method (Mæhre, Dalheim, Edvinsen, Elvevoll, and Jensen, 2018). The ground sample (200 mg) was loaded into the Dumas machine to determine the total nitrogen (N). The nitrogen content was measured with helium as carrier gas using an Elemental analyser Model Vario MAX CN Hanau, Germany. The protein percentage (dry basis) was converted from total nitrogen value as:

$$\text{Total protein (\%)} = N \times 6.25$$

Where, 6.25 is the correction factor used to convert total nitrogen content into crude protein content (Leser, 2013).

3.2.11 Fat content

Crude fat content was determined by AACC Method 30-10.01 (AACC, 2010c). Sample powder was placed in 500 ml beaker, added with 10 ml HCl 6N solution, and stirred (1 min) until dissolved. A 2 ml ethyl alcohol 96% was then added and mixed for 1 min. The beaker was placed in water (70-80°C) for 30 min. Intermittent shake was done every 10 min. A 10 ml ethyl alcohol (96%) was added and stirred well. A mixture than was cooled at room temperature. The mixture then was transferred to a separation funnel. A 25 ml ether in was added in three batches, followed by vigorously shaking (1 min). Each batch was rested for 3. A 25 ml petroleum ether was added in three batches with vigorously shaking (1 min) and was rested for 3 min between batches. The mixture then was stand for 20 min until clear supernatant formed. The supernatant was passed through filter papers 42 (125 mm diameter) into pre-weighed 500 ml round-bottom flask. It was then placed in steam bath in laboratory chamber to evaporate the ether. Obtained dry fat was placed in oven (100°C) for 100 min. The residue then was cooled and weighted. Crude fat was calculated as follow (Topete -Betancourt, 2020):

$$\text{Fat (\%)} = \frac{\text{weight of dry fat (corrected with blank)}}{\text{weight of sample}} \times 100$$

3.2.12 Ash content

Ash content was determined by AOAC official method for ash determination (AOAC, 2006a). Ground sample (5 g) was placed into pre-weighed crucible and placed in a furnace at 550°C overnight. The crucible was cooled down in a desiccator and then re-weighed. Ash content (%) was calculated based on following equation:

$$\text{Ash content (\%)} = \frac{\text{Ash weight}}{\text{Sample weight}} \times 100\%$$

3.2.13 Total dietary fibre content

Total dietary fibre (TDF) content was determined in duplicate using a Total Dietary Fibre assay kit K-TDFR-100A/K-TDFR-200A 08/16 (Megazyme Ltd., Wicklow, Ireland) based on AOAC (2006b) and AACC (2010a). Measurements were conducted for soluble dietary fibre (SDF) and insoluble dietary fibre (IDF) composition. The milled sample (1g) was placed into a 400 mL tall-form beaker with a magnetic stirrer bar and 40 mL MES-TRIS buffer (0.05 M, pH 8.2), and stirred. Heat-stable α-amylase (50 µL) was added and the mixture was incubated in shaking water bath at 98-100°C for 30 min. The beaker was scraped down with a spatula and rinsed with 10 mL water. Protease (100 µL) was added and the beaker was placed in a water bath at 60°C for 30 min. Then 5 mL 0.56 N HCI and 200 µL amyloglucosidase were added, and the beaker was incubated in a water bath at 60°C for 30 min. The content of beaker was poured into a sintered glass crucible with celite, suction was applied, and the residue was washed twice with 10 mL water at 70°C. The filtrate was transferred to a 600 mL tall-form beaker for SDF determination. The residue was washed twice with 10 mL of 95 % EtOH and twice with 10 mL acetone and labelled as IDF.

The saved filtrate had 4 volumes 95 % EtOH at 60°C added to it and was left at room temperature for 1 h and before being filtered through a sintered glass crucible with celite. The filtrate was washed with two 15mL portions of each of 78% EtOH, 95 % EtOH and acetone and labelled as SDF. Both IDF and SDF crucibles were dried in an oven at 103°C overnight and weighed. The dried fibre then was analysed for protein and ash.

3.2.14 Rapid visco analyser (RVA)

The finely ground sample (3.0 g) was placed into a canister and 25 mL distilled water was added. The pasting profiles were measured by a Rapid Visco Analyser (RVA) (Perten Instruments, Hagersten, Sweden) using standard profile 1, the sample was heated to 50°C and held for 1 min, then increased to 95°C and held for 1.5 min, before being cooled to 50°C and held for 2 min. The peak viscosity (PV), trough viscosity (TV), final viscosity (FV) and breakdown (BV) of materials were recorded (Ratnaningsih, Suparmo, Harmayani, and Marsono, 2020).

3.2.15 Total phenolic content and antioxidant capacities

3.2.15.1 Extraction of sample for total phenolic and antioxidant capacity

Extracts for the determination of total phenols and antioxidant capacity were prepared with a method described by Singleton and Rossi (1965). Pasta powder (2 g) was mixed with 20 mL of 70% methanol, kept in the multi-stirrer overnight at room temperature and then centrifuged at 2500 rpm (3000 g) for 10 min. The supernatant was collected into 10 mL plastic tubes and kept in -20°C until analysis.

3.2.15.2 Total phenolic content (TPC)

The TPC of the sample extracts was determined spectrophotometrically using Folin-Ciocalteau's (F-C) reagent according to the method described by Khanizadeh, Tsao, Rekika, Yang, and DeEll (2007); Lim and Murtijaya (2007a). The sample (500 µL) was added to the test tubes followed by 2.5 mL of 0.2 mol/L Folin-Ciocalteau reagent and 2.0 mL of sodium carbonate (7.5 g/100 mL). The contents of the tubes were mixed thoroughly and stored in the dark for 2 h before the absorbance was measured at 760 nm using VWR V-1200 Spectrophotometer (VWR International Co., Pennsylvania, USA). Gallic acid (0-200 µg) in methanol was prepared as a standard. TPC was expressed as mg gallic acid equivalents (GAE) per 100 g of dry matter (DM) material.

3.2.15.3 Ferric Reducing/Antioxidant Power (FRAP)

The FRAP was assessed according to Khanizadeh et al. (2007). A fresh working solution of FRAP reagent was prepared each time by mixing acetate buffer (300 µM, pH 3.6), a solution of 10 mM TPTZ in 40 mM HCL, and 20 mM $FeCl_3 \bullet 7H_2O$ at 10:1:1 (v/v/v). A standard of iron (II) sulphate ($FeSO_4 \bullet 7H_2O$) (0-200 µmol) or sample extract (250 µL) was added to 2.5 mL of the FRAP reagent and the absorbance at 593 nm was recorded immediately after the addition of the sample and again after 2 h incubation at 37°C (Le, 2012). The results were expressed as mmol Fe^{2+}/100g sample.

3.2.15.4 ABTS (2,2' -azino-bis (3-ethylbenzothiazoline-6-sulfonic acid)) radical scavenging capacity

The ABTS radical scavenging assay used in this thesis was based on the method of Elfalleh et al. (2009). An $ABTS^{\bullet +}$ reagent was prepared by reacting colourless ABTS stock solution (7mM in water) with 2.45 nM potassium persulfate and allowing the reaction to stand for 16 hours in the dark at room temperature. On the day of analysis, the $ABTS^{\bullet+}$ solution was diluted with PBS (pH 7.4) to an absorbance of 0.70 (± 0.02) at 734 nm and 3 mL transferred to a cuvette. After the addition of 300 µL Trolox (0-200 µmol) or sample extract, the mixture was well mixed, allowed to stand for 6 min and the absorbance was read at 734nm. The results were expressed as µmol Trolox equivalents (TE) per 100 g DM material.

3.2.16 Amino acid profiles

Amino acid (AA) profiles were determined using Agilent 1100 series (Agilent Technologies, Walbronn, Germany) high-performance liquid chromatography following the methodology by Heems, Luck, Fraudeau, and Vérette (1998). Before injected, the sample was hydrolysed with 6 N hydrochloric acid in an oven at 110°C for 20 h. The HPLC was equipped with a 150 x 4.6 mm, C18, 3uACE-111-1546, (Winlab, Glasgow, Scotland) column for amino acid separation. Column flow rate was 0.7 mL/min, and the temperature was kept at 40°C. Primary amino acids were detected using O-phthaldialdehyde (OPA) as a fluorescence derivative reagent, while secondary amino acids were using 9-fluorenylmethyl chloroformate (FMOC). Detection utilised a fluorescence detector with an excitation of 335 nm and emission of 440 nm for primary amino acids. The detector was switched at 22 min to excitation 260 nm, emission 315 nm to detect secondary amino acids such as proline. The amino acid results are expressed in mg of amino acids per g protein.

3.2.17 Colour measurement

Colour readings of cooked and uncooked pasta were taken using a Tristimulus Colour Analyser (Minolta Chroma meter CR 210m, Minolta Camera Co., Japan). Results was expressed as L* (brightness), a*(redness) and b*(yellowness) (Foschia, Peressini, Sensidoni, Brennan, and Brennan, 2015a).

3.2.18 Cooking properties of pasta

3.2.18.1 Optimum cooking time (OCT)

Pasta strands (20 g) were cut into 40 mm lengths and cooked in 300 mL of boiling water. A sample pasta strand was taken every 30 second during cooking and squeezed between two transparent glass slides. The time at which the disappearance of the core of pasta was observed was recorded as the OCT (AACC, 2010b).

3.2.18.2 Cooking loss (CL)

A pasta sample (10 g) was cooked in 600mL of boiling water for OCT and rinsed with 100 mL of cold water. Cooked pasta was used for swelling index and water absorption index determination. The cooking water was collected in an aluminium vessel, placed in an air oven at 105°C and evaporated until constant weight reached. The residue was weighed, and the cooking loss was reported as a percentage of starting material.

3.2.18.3 Swelling index (SI) and water absorption index (WAI)

The SI of cooked pasta (g water/g dry pasta) was determined according to the procedure described by Brennan and Cleary (2005). Pasta (10 g) was weighed after cooking and dried at 105°C until constant weigh reached. The result was calculated as:

$$\mathrm{SI} = \frac{Wc - Wd}{Wd}$$

where:

Wc : weight of cooked pasta (g)

Wd : weight of pasta after drying (g)

The water absorption index (g/ 100 g) was determined as:

$$WAI = \frac{Wc - Wr}{Wr} \times 100$$

where:

Wc : weight of cooked pasta (g)

Wr : weight of uncooked pasta (g)

3.2.19 Textural properties

The texture of the cooked pasta was determined using a Texture Analyzer (TA.XT2, Stable Micro System, UK) equipped with a 5 kg load cell. Samples were cooked for their OCT and rested for 10 min, and then tested. Firmness and extensibility of the cooked pasta were determined according to the method described by Foschia et al. (2015a). Firmness was measured by placing five strands of cooked pasta parallel to each other on a flat metal plate then compressing them using a blade. Extensibility was measured by placing a pasta strand around the A/SPR spaghetti/noodle rig and then applying tension (settings: pre-test speed was 3 mm/s; test speed was 3 mm/s; post-test speed was 5 mm/s; initial distance was 10 mm; final distance was 100 mm). Data were collected from three different cooking replications with four replications of each (n=12).

3.2.20 *In vitro* starch digestibility

Pasta samples (20 g) were cooked in boiling tap water (600 mL) for their OCT and cut with knife to obtain a 2-5 mm size. The reducing sugars released over 120 min were evaluated for each pasta type as described by Foschia et al. (2015a).

A 2.5 g sample was weighed into a plastic pottle with 30 mL of RO water then placed on a pre-heated magnetic heater stirring block (IKAAG RT 15, IKA-WERKE Gmbit & Co., Staufen, Germany) and held at 37°C with constant stirring. To the mixture 0.8 mL 1M HCl and 1 mL of 10 % pepsin (Sigma Aldrich, USA) solution in 0.05 M HCl was added with continuous stirring and incubated at 37°C for 30 min. An aliquot (1 mL) was taken (time 0) and added to 4 mL ethanol in 15 mL Falcon plastic tube. Amyloglucosidase (0.1 mL) was added to the digestion pot. Enzyme solution (5 mL of 2.5% Pancreatin (Sigma Aldrich, USA) in 0.1 M sodium maleate buffer pH 6) was added with constant stirring at 37°C for 120 min and the aliquot (1 mL) were taken at 20, 60 and 120 min and added to 4 mL ethanol. The samples were stored at 4°C.

The plastic tubes containing the sample aliquot were centrifuged at 1000 g for 10 min. An aliquot (0.05 mL) of each was placed in glass test tube alongside the reagent blank (0.05 mL RO water), 0.05 mL of 5 mg/mL glucose standard and 0.05 mL 10 mg/mL tubes. An enzyme solution (0.25 mL) of 1 % Invertase and 1% amyloglucosidase in acetate buffer pH 5.2 was added to glass tube and kept for 20 min at room temperature before 0.75 mL of the DNS (dinitro-salicylic) reagent was added to each tube. The tube then was covered with aluminium foil and heated for 10 min in a boiling water bath. The glass tubes were cooled before adding 4 mL of distilled water and the absorbance was read at 530 nm against distilled water. Reducing sugar release was calculated as mg /g sample, area under the curve (AUC) was calculated against the time.

3.2.21 *In vitro* protein digestibility

In vitro protein digestibility of cooked pasta was performed using a multi-enzyme technique (Desai, Brennan, and Brennan, 2018). A 50 mL of protein suspension was prepared in distilled water (6.25 mg of protein/mL). Suspension then was adjusted to pH 8 with a solution of 0.1 N HCL and /or 0.1 N NaOH, and placed on magnetic heating stirring block at 37°C. The multi-enzyme solution (1.6 mg/mL Trypsin, 3.1 mg/mL chymotrypsin and 1.3 mg/mL protease) was kept in an ice bath and adjusted to pH 8.0. Five mL of the multi-enzyme solution was then added to the protein suspension, which was maintained at 37°C. The decrease in pH then was observed every minute for period of 10 minutes using a digital pH meter (S20 Seven Easy™, Mettler Toledo, USA). The percent protein digestibility (Y) was calculated by using equation:

$$Y = 210.46 - 18.10\,X$$

Where X represents the change in pH after 10 min.

3.2.22 Amino acid score and protein digestibility corrected amino acid score

Amino acid scores (AAS) were calculated by dividing the amino acid content of the sample (mg/g protein) by the suggested reference pattern of amino acid requirements (mg/g protein) for pre-school children (1–2 years old) for nine essential amino acids plus tyrosine and cysteine as follows: histidine—18, isoleucine—31, leucine—63, lysine—52, methionine + cysteine—25, phenylalanine + tyrosine—46, threonine—27, and valine—41 (WHO, FAO, and UNU, 2007). The protein digestibility corrected amino acid score (PDCAAS) was calculated by multiplying the corresponding protein digestibility percentage by the lowest AAS value. PDCAAS values exceed 1.00 were truncated to 1 (FAO, 2013).

3.2.23 Sensory analysis

Sensory analysis was determined using an affective quantitative method according to Zandonadi et al. (2012) and Gao, Brennan, Mason, and Brennan (2017). The trial was conducted under approval permission of Lincoln University human ethic committee. Sensory tests were using a hedonic scale of nine points (9_extreme like to 1_extreme dislike). The number of consumer panel were 37 untrained panellists (staff and student of Lincoln University) who are not averse to pasta product. The attributes were appearance, aroma, flavour, texture, and overall quality of the product. Cooked samples include control were coded with 3-digit random numbers and served on white plastic plate at the same time. Panellist received 20 g of each cooked pasta sample and was advised to drink water at room temperature (approximately 25°C) between the observation of each sample.

3.2.24 Statistical analysis

All experiments were conducted in triplicates unless otherwise stated. Minitab 18.0 (Minitab Pty Ltd, New South Wales, Australia) was used to analyse ANOVA (analysis of variance) of one-way and general linear model.

3.2.24.1 One-way ANOVA

One-way ANOVA was used to compare all treatments and against control (semolina flour/pasta), followed by Tukey's comparison test ($p < 0.05$).

3.2.24.2 General linear model ANOVA

General linear model ANOVA was performed to determine the effects of two factors applied in the experiment (type of protein and level of protein) with control (semolina pasta) excluded.

Tukey's comparison test ($p < 0.05$) was performed to determine significant difference between factors.

Chapter 4

Physico-Chemical, Pasting Properties and Nutritional Quality of Banana Flour and Cassava Flour in Comparison to Semolina Flour

This chapter is submitted as:

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Abstract

This study evaluated the proximate composition, amylose, resistant starch, pasting properties, total phenolic content (TPC), antioxidant capacities, and amino acid profiles of banana flour and cassava flour in comparison to semolina flour. Banana flour and cassava flour had a lower protein content (4.54 and 1.41%) but higher total dietary fibre (16.46 and 10.99%) than semolina flour (12.36 and 7.07%, respectively). The two gluten-free flours had a lower amylopectin content compared to semolina flour. Banana flour showed high nutritional qualities (resistant starch, TPC, and antioxidant capacities) compared to cassava and semolina flour. The amino acid evaluation showed that banana and cassava flour had a better ratio of total essential amino acid and total amino acid (35.37 and 29.95% versus 23.34%) but had lower limiting essential amino acid values (0.98 and 1.51% versus 11.12%) than semolina flour. Different physico-chemical, functional, and nutritional properties of banana and cassava flour showed potential information in the development of gluten-free cereal products based on these flours.

Keywords: *banana flour, cassava flour, physico-chemical, pasting, amino acid, gluten-free*

4.1 Introduction

Increasing awareness of coeliac disease around the world, and high economic cost of wheat in some regions, has led to many studies to find sustainable and alternative materials for wheat (Odey and Lee, 2020). Consumers are consciously choosing health food sources to combat the increasing incidences of chronic diseases such as diabetes, obesity, and cardiovascular diseases (Campuzano et al., 2018; Kumar et al., 2019). Gluten-free food have become part of the new healthy lifestyle trend with significant increasing demand over the years (Woomer and Adedeji, 2020).

Among the food functional option, one of the ideal carriers to deliver the interest of healthy food due to its global consumption, cheap cost, and long shelf life is pasta product (Nilusha, Jayasinghe, Perera, and Perera, 2019). Common pasta is made from semolina that is produced from durum wheat (*Triticum turgidum L. ssp. durum*) (Parada et al., 2020). Semolina and pasta colour come from two colour pigments, yellow (desirable) and brown (undesirable). The yellow colour comes from carotenoid accumulation in kernels, which also consider as an antioxidant source. The use of semolina for pasta materials is mainly for the commercial value since a bright yellow colour is preferable in pasta products (Colasuonno et al., 2019). It has been also reported that durum wheat semolina has a high protein content and gluten strength, leads to its visco-elasticity that essential to make pasta product with good firmness, low stickiness, and bulkiness (Cecchini, Antonucci, Costa, Marti, and Menesatti).

In recent year, there are many efforts in the food industry to develop pasta products with additional ingredients to enhance the nutritional qualities. Incorporation of dietary fibre, vitamins, minerals, natural pigments, and antioxidants have been reported to improve the functional properties of conventional pasta products (Nilusha et al., 2019). Another interest also has been developed in providing healthy food sources regarding coeliac disease and other gluten-related disorder (Trevisan et al., 2019). Various alternative materials that can be used as the substitution of wheat flour to develop gluten-free products including gluten-free pasta. However, it becomes quite challenging to choose gluten-free materials that meet with consumer related desirable food products characteristics (Patil, Sonawane, Mali, Mhaske, and Arya, 2020). Among the potential ingredient sources, banana flour and cassava flour may be the promising alternatives raw materials based on those wide availability and the large yield production (Kumar et al., 2019; Ramirez et al., 2019).

The application of banana flour and cassava flour in food products is due to their versality, low-cost food source, and high calorie source (high starch content) (Borges et al., 2020; Odey and Lee, 2020). Many researchers have reported physico-chemical properties and nutritional values of banana flour as well as functional properties related to product characteristics. Banana flour has been proven to have high dietary fibre and resistant starch content, with a specific pasting and textural properties (Campuzano et al., 2018; Cheok et al., 2018; Kumar et al., 2019). Unlike banana flour, studies on cassava flour characterisation are still limited to lack of research regarding the basic physico-chemical properties and technical properties (pasting and textural properties) (Odey and Lee, 2020; Ramirez et al., 2019).

The initial challenge in gluten-free product development is to provide comprehensive information of gluten-free materials. This can be fundamental support in making consideration of abundant gluten-free materials utilisation. The characterisation of gluten-free material is important to illustrate their rheological and functional properties that play important role to the quality and characteristics of final products (Patil et al., 2020).

Various studies have reported the development of gluten-free products, especially in gluten-free pasta areas that involving modification of raw materials and processing technologies. However, to the best authors knowledge, no study has been addressed on characteristics of alternative gluten-free flours that will be useful in the development of gluten-free pasta with regards to semolina flour as well as those nutritional qualities that may be added values.

Therefore, the objective of the study was to evaluate the physico-chemical (total starch, amylose-amylopectin, protein, fat, ash, dietary fibre), pasting properties, nutritional values including resistant starch and amino acid profiles of banana flour and cassava flour as gluten-free materials especially in pasta product in comparison with semolina flour.

4.2 Materials and methods

4.2.1 Raw materials

Banana flour, cassava flour, and semolina flour as described in section 3.1.

4.2.2 Moisture content

Described in section 3.2.6.

4.2.3 Carbohydrate content

Described in section 3.2.7.

4.2.4 Amylose-amylopectin content

Described in section 3.2.8.

4.2.5 Resistant starch content

Described in section 3.2.9.

4.2.6 Protein content

Described in section 3.2.10.

4.2.7 Fat content

Described in section 3.2.11.

4.2.8 Ash content

Described in section 3.2.12.

4.2.9 Dietary fibre content

Described in section 3.2.13

4.2.10 Pasting properties

Described in section 3.2.14

4.2.11 Total phenolic content and antioxidant capacities

Described in section 3.2.15

4.2.12 Amino acid profiles

Described in section 3.2.16

4.2.13 Statistical analysis

Described in section 3.2.24 and 3.2.24.1.

4.3 Result and discussion

4.3.1 Physico-chemical properties of banana and cassava flour in comparison to semolina flour

The moisture content, total starch, protein, fat, ash and dietary fibre of banana, cassava and semolina flour can be seen in table 4.1.

Table 4.1 Proximate analysis of banana and cassava flour in comparison to semolina flour

Component (% dry basis)	Banana	Cassava	Semolina
Moisture	8.50 ± 0.10^{c}	12.16 ± 0.08^{b}	17.48 ± 0.02^{a}
Protein	4.54 ± 0.03^{b}	1.41 ± 0.03^{c}	12.36 ± 0.03^{a}
Fat	0.44 ± 0.02^{b}	0.34 ± 0.00^{c}	0.94 ± 0.01^{a}
Insoluble dietary fibre (IDF)	12.91 ± 0.35^{a}	9.49 ± 0.19^{b}	5.42 ± 0.01^{c}
Soluble dietary fibre (SDF)	3.55 ± 0.16^{a}	1.51 ± 0.29^{b}	1.65 ± 0.43^{b}
Total dietary fibre (TDF)	16.46 ± 0.19^{a}	10.99 ± 0.11^{b}	7.07 ± 0.44^{c}
Ash	3.51 ± 0.01^{a}	0.85 ± 0.03^{b}	0.49 ± 0.03^{c}

Mean ± standard deviation. Values within a row followed by the same superscript letter are not significantly different from each other ($p > 0.05$).

The most significant differences between cassava and banana flour compared to semolina flour are protein content and TDF content. Semolina was observed to have a significantly higher protein content (12.36%) than banana flour and cassava flour (4.54 and 1.41%, respectively). This is a disadvantage composition since adequate protein is needed in gluten-free product development, both in improving textural quality and nutritional content (Woomer and Adedeji, 2020). Hence, this characteristic should be evaluated when utilising these flours to produce gluten-free cereal products.. Simple efforts can be implemented to improve the food product quality based on the gluten-free flour such as by adding the functional ingredients or additives (protein, gums, hydrocolloids, and emulsifier) (Gao et al., 2018). Many studies have reported the lack of protein in banana flour and cassava flour (Kumar, Brennan, Zheng, and Brennan, 2018; Oluba et al., 2017). However, no study has defined the protein quality of these flours that further discuss later in amino acid profiles section.

The moisture content of banana flour and cassava flour are much lower compared to semolina pasta. Moisture content depends on the drying conditions that may influence the final product. Ramirez et al. (2019) reported that minimising hot-air drying time during cassava flour processing led to better texture properties of gluten-free pasta. The fat content of all flours was relatively low (less than 1%) with cassava flour having the lowest fat content (0.34%). Banana flour had a significant ash content which showed a higher mineral content compared to cassava flour and semolina flour. Similar ash content of banana flour (1.63 – 2.61%) was reported in couple studies and related with significant micronutrients content such as kalium (600 – 1300 mg/100g) and phosphorus (112 – 252 mg/100g) (Borges et al., 2020; Campuzano et al., 2018).

The TDF on the other hand was found to be higher in banana and cassava flour than in semolina pasta. Banana had the highest TDF (16.46%) compared to cassava (10.99%) and semolina flour (7.07%). High dietary fibre content promoted positive effect on nutritional properties of cereal products such as decreasing glycaemic index, however it is also altered to a poorer quality and consumer acceptance of the final products (Foschia et al., 2015a; Foschia, Peressini, Sensidoni, Brennan, and Brennan, 2015b). Banana flour had a higher soluble dietary fibre content compared to cassava flour that has beneficial for intestinal such as reducing constipation (de Angelis-Pereira, Barcelos, Pereira, Pereira, and de Sousa, 2016).

Total starch content of cassava flour was higher compared to semolina flour, while banana flour had the lowest value (Table 4.2). A high starch content showed a promising ingredient to develop into food products, while high protein content led to better nutritional quality and better functional properties, such as higher surface charge, better starch swelling and affecting starch gelatinisation (Huang, Martinez, and Bohrer, 2019). As the major component of flour is carbohydrate, it is also necessary to evaluate amylose content and resistant starch. Amylose content and resistant starch (RS) content of three different flours varied as shown in table 4.2.

Table 4.2 Amylose and resistant starch content of banana, cassava, and semolina flour

Component	Banana	Cassava	Semolina
Total starch (% dry basis)	73.36 ± 0.57^{b}	82.30 ± 1.82^{a}	77.20 ± 2.25^{a}
Amylose (% of total starch)	19.04 ± 0.99^{b}	17.96 ± 0.25^{b}	30.67 ± 0.77^{a}
Resistant starch (% dry basis)	52.75 ± 0.71^{a}	1.52 ± 0.14^{c}	6.18 ± 0.61^{b}

Mean ± standard deviation. Values within a row followed by the same superscript letter are not significantly different from each other ($p > 0.05$).

Banana and cassava flour had similar amylose content, while semolina flour had significant higher amylose content. The amylose content in flour may contribute to physico-chemical of gluten-free cereal products. In gluten-free bread, especially when low protein ingredients took place, a high amylose content may promote the crumb structure formation as it retrogrades faster compared to amylopectin (Roman, Reguilon, Gomez, and Martinez, 2020). In pasta making, high amylose content contributes to low cooking lost, low stickiness, high firmness, and high chewiness, which showed that low amylose content tends to produce low quality pasta (Cordelino et al., 2019). This characteristic illustrated that despite the absence of gluten which plays important role of the quality of the final cereal products, banana and cassava flour might produce inferior quality because of its lower amylose content compared to semolina pasta.

The RS content of banana flour (52.75%) was significantly higher compared to semolina flour (6.18%), while cassava flour had the lowest value (1.52%). High RS content in banana flour agrees with previous studies suggesting high RS content in this flour (Borges et al., 2020; Campuzano et al., 2018; Kumar et al., 2019). Cassava flour has been reported to have low RS content (1.12 – 4.14%) (Chisenga, Workneh, Bultosa, and Laing, 2019; Pereira and Leonel, 2014). Higher RS content in the mixture ingredient improved nutritional values by decreasing glycaemic index and mineral bioavailability, but at the same time deteriorated the quality (lower firmness, higher cooking loss) of pasta products (Aribas, Kahraman, and Koksel, 2020; Garcia-Valle et al., 2019).

4.3.2 Pasting properties banana and cassava flour in comparison to semolina flour

Table 4.3. Shows pasting properties of banana and cassava flour compared to semolina flour. Pasting properties varied among flours due to different characteristics such as shape, size, rigidity, functional properties, and amylose content (Patil et al., 2020).

Table 4.3 Pasting properties of banana, cassava, and semolina flour

Parameter	Banana	Cassava	Semolina
Peak viscosity (cP)	1107.00 ± 6.00^{b}	2692.3 ± 47.0^{a}	742.3 ± 100.8^{c}
Breakdown viscosity (cP)	$136.33\ 6.66^{b}$	$785.30\ 32.70^{a}$	$34.33\ 5.69^{c}$
Final viscosity (cP)	1712.33 ± 13.58^{b}	2514.67 ± 30.35^{b}	1537.00 ± 137.49^{c}
Setback viscosity (cP)	741.67 ± 16.92^{b}	607.67 ± 22.74^{c}	829.00 ± 40.78^{a}
Peak Time (min)	7.00 ± 0.00^{a}	4.98 ± 0.04^{b}	6.53 ± 0.81^{a}
Pasting Temp (°C)	82.30 ± 0.05^{b}	76.17 ± 0.46^{c}	89.88 ± 1.24^{a}

Mean ± standard deviation. Values within a row followed by the same superscript letter are not significantly different from each other ($p > 0.05$).

Banana flour and cassava flour had higher values in almost all point of viscosities (peak viscosity (PV), breakdown viscosity (BV), and final viscosity (FV)) compared to semolina pasta. Cassava flour showed a high breakdown viscosity (BV) (785.30 cP) which indicates it tend to break down easier during cooking (Patil et al., 2020). Semolina flour had a higher value of setback viscosity (SBV) (829 cP) compared to banana and cassava flour (741.67 and 607.67 cP, respectively). SBV indicates the retrogradation ability of flour and may be affected by other components including dietary fibre, protein, lipid and polyphenols (Marta, Cahyana, Djali, Arcot, and Tensiska, 2019; Patil et al., 2020). Pasting temperature of semolina flour (89.88 °C) was also higher than banana and cassava flour (82.30 and 76.17 °C, respectively) which showed banana and cassava flour need a lower temperature to cook compared to semolina flour (Huang et al., 2019).

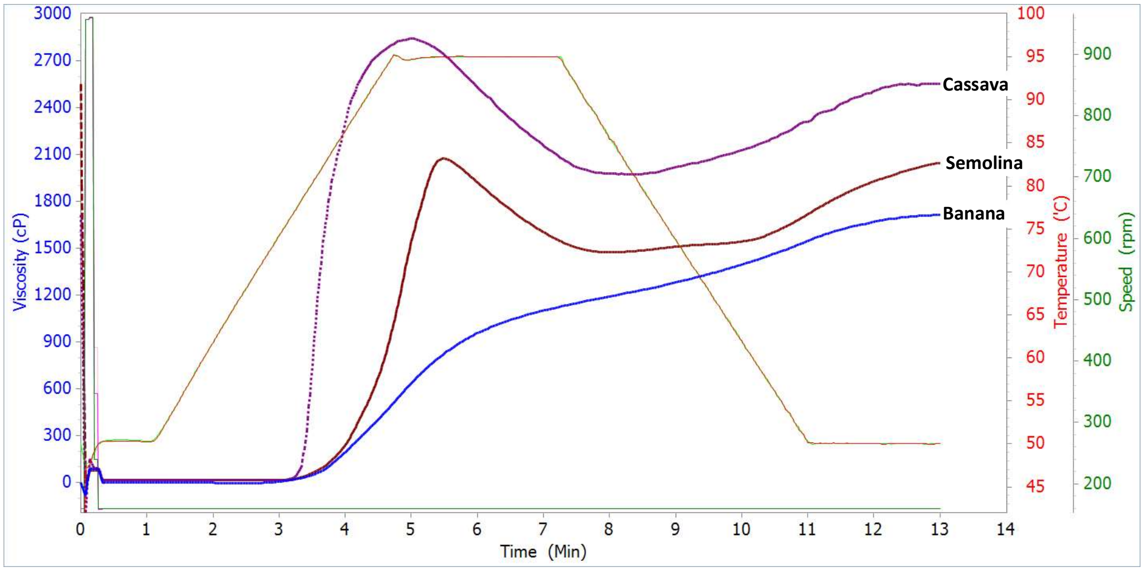

Figure 4.1 Pasting profiles of banana, cassava, and semolina flour

It is noticeable in figure 4.1 that banana flour had a different profile of pasting, where well-developed peak did not occur. The viscosity of banana flour did not reach the top in any point after maximum temperature (95°C) had been reached out in standard 1 RVA profile. This showed that banana flour did not swell completely and tent to have resistance to swelling and heating at 95°C. This pattern also found in previous studies regarding banana flour pasting profiles (Cheok et al., 2018; Huang et al., 2019). Banana flour showed a well-developed peak curve and reached higher peak viscosity (4142.00 cP) when standard 2 RVA with maximum temperature 130°C applied, much higher than wheat flour (1597.00 cP) at the same standard RVA (Huang et al., 2019).

The correlation between pasting properties and physicochemical characteristics are shown in table 4.4 illustrates some physico-chemical composition affected the pasting properties significantly.

Table 4.4 Correlation between physico-chemical composition and pasting properties of flours

Parameter	Moisture	Total starch	Protein	Fat	Ash	Amylose	RS
PV	-0.258	0.748*	-0.829**	-0.753*	-0.236	-0.694*	-0.416
TV	-0.289	0.72*	-0.845**	-0.771*	-0.204	-0.716*	-0.384
BV	-0.208	0.787*	-0.8**	-0.72*	-0.285	-0.656*	-0.462
FV	-0.248	0.738*	-0.817**	-0.738*	-0.242	-0.678*	-0.419
SBV	0.452	-0.581	0.906**	0.859**	0.014	0.831**	0.195
Peak T	-0.125	-0.762*	0.504	0.398	0.548	0.344	0.677
Pasting T	0.616	-0.471	0.977**	0.945**	-0.167	0.91**	0.021

***P-value < 0.01, *P-value < 0.05*

Protein content was the most affecting parameter on whole pasting characteristics, followed by fat, amylose, and total starch content. PV, TV, and FV were decreased significantly by the increase of protein, fat, and amylose content, while these values increased by a higher total starch content. On the contrary, SBV increased by a higher composition of protein, fat, and amylose content and reduced by carbohydrate content. Moisture, ash, and resistant starch content did not have any correlation with pasting properties that showed these parameters did not give an effect on pasting properties. The higher PV and FV, and lower SBV by a lower protein, amylose and fat content in flours observed in waxy starches, including maize, rice, wheat, and oat flour (Hsieh, Liu, Whaley, and Shi, 2019; Li et al., 2020; Oñate Narciso and Brennan, 2018; Shevkani, Singh, Kaur, and Rana, 2014). The lack of amylose content inhibits the swelling of granules during heating (Hsieh et al., 2019), while protein prevent the amylose molecules reformation and retrograde during cooling resulting on lower final viscosities (Oñate Narciso and Brennan, 2018). The lipid-starch interaction can form strong complexes bond that slowing down the swelling process and decreased the paste viscosities (Shevkani et al., 2014).

4.3.3 Total phenolic content and antioxidant activities of banana and cassava flour in comparison to semolina flour

A clear comparison of TPC, FRAP, and ABTS of banana, cassava and semolina flour can be seen in table 4.5. TPC, ABTS and FRAP values of banana flour met agreement with similar research of banana flour (103 -160 mg GAE/100 g, 0.2 -3.6 mmol/100 g, 0.6-39.7 mmol Fe/100g, respectively) (Borges et al., 2020; Kumar et al., 2019). There is scarce research that evaluate the TPC and antioxidant activities of cassava four and this flour has been known to have lack of nutritional quality (Odey and Lee, 2020).

Table 4.5 TPC and antioxidant capacities of banana, cassava, and semolina flour

Parameter	Banana	Cassava	Semolina
TPC (mg GAE/100 g DM)	116.45 ± 4.75^{a}	46.69 ± 1.20^{c}	73.80 ± 0.78^{b}
ABTS (µmol TE/100 g DM)	1.46 ± 0.02^{a}	0.87 ± 0.02^{b}	0.67 ± 0.02^{c}
FRAP (mmol Fe/100 g DM)	1.14 ± 0.00^{a}	0.54 ± 0.01^{b}	0.15 ± 0.02^{c}

Mean ± standard deviation. Values within a row followed by the same superscript letter are not significantly different from each other ($p > 0.05$).

Banana flour had the highest of all TPC and antioxidant activities, while cassava showed poorer values compared to semolina pasta. The superior TPC and antioxidant capacities of banana flour made it as a source of TPC and antioxidant to be added in food products (Biernacka, Dziki, Rózyło, and Gawlik-Dziki, 2020; Martins et al., 2019). In contrast, some studies have been done to enhance phenolic content and antioxidant activities of cassava-based food products by incorporate high phenolic and antioxidant properties ingredients, one of which was banana flour (Irondi, Awoyale, Oboh, and Boligon, 2019; Wang, Zhang, and Mujumdar, 2012).

4.3.4 Amino acid profiles of banana, cassava, and semolina flour

Amino acid profiles of the three flours can be seen on table 4.6. In general, banana and cassava flour had higher ratio of total essential amino acid and non-essential amino acid (TEAA/TAA).

Table 4.6 Amino acid profiles of banana, cassava, and semolina flour (mg/100 g protein)

Amino acid	Banana flour	Cassava flour	Semolina flour
Essential amino acid			
Valine	11.86 ± 1.44^{b}	11.04 ± 1.29^{b}	30.35 ± 2.22^{a}
Methionine	0.98 ± 0.96^{b}	1.51 ± 0.68^{b}	11.12 ± 1.04^{a}
Phenylalanine	14.64 ± 1.01^{b}	11.97 ± 0.90^{b}	40.83 ± 3.09^{a}
Isoleucine	5.06 ± 5.57^{b}	4.75 ± 1.01^{b}	25.23 ± 1.94^{a}
Lysine	119.10 ± 1.87^{a}	95.38 ± 1.58^{b}	50.33 ± 4.59^{c}
Leucine	18.59 ± 1.02^{b}	14.27 ± 1.61^{b}	55.55 ± 4.02^{a}
Histidine	136.63 ± 1.18^{a}	95.12 ± 3.77^{b}	59.17 ± 4.92^{c}
Threonine	60.04 ± 6.71^{a}	45.68 ± 0.57^{b}	35.85 ± 2.82^{c}
Non-essential amino acid			
Aspartic acid	133.19 ± 1.62^{a}	74.68 ± 4.14^{b}	45.24 ± 1.97^{c}
Glutamic acid	160.37 ± 1.14^{b}	170.70 ± 4.54^{b}	394.31 ± 13.28^{a}
Cysteine	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}	191.62 ± 14.45^{a}
Serine	116.59 ± 1.93^{a}	85.95 ± 1.59^{b}	68.02 ± 6.78^{b}
Arginine	130.54 ± 1.04^{b}	214.81 ± 8.56^{a}	65.78 ± 5.42^{c}
Alanine	37.87 ± 3.21^{a}	30.46 ± 1.14^{b}	29.54 ± 2.15^{b}
Tyrosine	12.83 ± 1.04^{b}	14.30 ± 0.71^{b}	21.75 ± 1.77^{a}
Glycine	49.24 ± 4.81^{a}	35.75 ± 0.52^{b}	35.77 ± 1.67^{b}
Proline	29.59 ± 2.14^{b}	27.63 ± 3.24^{b}	112.54 ± 2.37^{a}
Total AA	1037.12 ± 61.60^{b}	934.00 ± 31.27^{c}	1320.35 ± 38.84^{a}
TEAA/TAA (%)	35.37 ± 0.31^{a}	29.95 ± 0.09^{b}	23.34 ± 0.92^{c}

Mean ± standard deviation. Values within a row followed by the same superscript letter are not significantly different from each other ($p > 0.05$).

Essential amino acid (EAA) in banana flour was dominated with histidine, lysin and threonine (136.63, 119.10, and 60.04 mg/100 g protein, respectively), similar with cassava with lower values (95.12, 95.38, and 45.68 mg/100 g protein). It also should be noted that these two gluten-free flours had limiting value of EAA, which is methionine (0.98 – 1.51 mg/100 g protein). Glutamic acid was the most abundant amino acid of banana flour that in agreement with other studies (Famakin, Fatoyinbo, Ijarotimi, Badejo, and Fagbemi, 2016; Lee et al., 2019). Unlike banana and cassava flour, semolina flour had better dispersion of EAA, with limiting EAA value of 11.12 mg/100 g protein (methionine). Amino acid profiles can be a basic information to develop nutritional value of food products. The effort to obtain better amino acid profile can be achieved by combining with other protein resources through it limiting AA and amino acid profiles (Bezerra, Rodrigues, Amante, and Silva, 2013).

4.4 Conclusion

The results of this study illustrate the differences of physico-chemical, functional, and nutritional properties of banana and cassava flour as potential gluten-free flours compared to semolina flour. Cassava and banana flour were inferior in most of physico-chemical and functional characteristics as pasta ingredients but had the advantage in terms of starch and protein nutritional properties.

Chapter 5

Effect of Cassava and Banana Flours Blend on Physico-Chemical Characteristics and Glycaemic Properties of Gluten-Free Pasta

This chapter is published as:

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Abstract

Cassava flour and banana flour were used in a variety of ratios (0:100, 25:75, 50:50) to produce gluten-free (GF) pasta, the physico-chemical and glycaemic properties were investigated and compared to a control pasta made from durum wheat semolina. All GF pasta had a higher dietary fibre content, lower protein content, and a darker colour compared to the control. The optimum cooking time (2.8-4.2 min) and water absorption index (23.28-86.81%) of the GF pastas were lower than the control, while cooking loss was higher (15.18-28.75%). The best formulation of GF Pasta among the blend ratios was achieved from 100% banana flour. GF pasta containing predominantly banana flour (75:25 banana : cassava flour and 100% banana flour) had a reduced predictive glycaemic loading. The results illustrate that cassava and banana flours can be considered as suitable materials to develop gluten-free pasta.

Keywords: *Cassava flour, banana flour, gluten-free pasta, dietary fibre, glycaemic index*

5.1 Introduction

Conventional pasta is made of durum wheat semolina and water through an extrusion process (Hager et al., 2012). The high level of gluten in semolina enables the production of a strong and elastic dough. Semolina also has a high level of yellow pigment and low lipoxygenase activity making it suitable for pasta manufacture. In addition, semolina has uniformly fine particles which contribute to hydration properties and high quality pasta. These characteristics make semolina flour a superior material for pasta production. However, different types of cereal flours are being utilized for pasta production due to market trends, that demand high nutritional characteristics, together with the development of alternative raw materials for gluten-free pasta. Unfortunately, the gluten-free flours (maize, rice, millet, and sorghum) cannot create a sufficiently strong binding network to produce the required quality of dough. Hence, it is necessary to improve the pasta making process, or develop new formulation with additional ingredients, in order to make adequate sensory and cooking quality of pasta products (Padalino et al., 2016).

The most common gluten-free pasta raw materials are rice and corn. Hager et al. (2012), found that rice was used in 22 out of 33 commercial gluten-free pasta products, while corn has been used in 20 out of 33 gluten-free pasta products. There are still a lot of gaps between research and commercial use, such as amaranth flour which has been used in over 20 research projects but is only found in 1 commercial pasta product. Other commercial products use buckwheat and quinoa as base or addition ingredients (Hager et al., 2012). Marti et al. (2013) mentioned potato as a common use material in gluten-free pasta making, but there is little research about potato utilization in gluten-free pasta making.

Alternative raw materials that have potential to be used in gluten-free pasta making are cassava and banana flours due to their carbohydrate content and commercial availability. The annual yield of cassava and banana in 2014 are 6th and 8th respectively in world crop production, with volumes of 260 billion kg and 114 billion kg, respectively. This production provides a potential for development as base materials for food products, compared to wheat, rice and corn which top the annual world crops production, with volumes of 729 billion kg; 741 billion kg; and 1 trillion kg respectively (Food and Agriculture Organization, 2017).

Previous research has suggested that cassava and banana may be utilized successfully in pasta making as either an additional ingredient or even as the base material. However, there are some limitations since these materials have no gluten content. Some efforts have been made in material application with modification of raw material pre-treatment, processing technology and technical addition to replace gluten functionality (Baah et al., 2005); Fiorda et al. (2013a); Leonel et al. (2011); Sarawong, Schoenlechner, Sekiguchi, Berghofer, and Ng (2014b).

Most of research in gluten-free pasta has succeeded in creating a desirable gluten-free pasta, some of them also stated the nutritional values of pasta products, but none of them investigated how the ingredients affect nutritional properties in the final product. This may be because the use of the gluten-free materials gives the benefit of being gluten-free, however, it is also disadvantageous since most alternative materials provide lower nutritional values than wheat or other flours. The objectives of the research were to investigate the utilization of cassava and banana and those effects on physical and glycaemic properties of gluten-free pasta.

5.2 Material & methods

5.2.1 Raw material

Described in section 3.1.

5.2.2 Raw pasta preparation

Described in section 3.2.1. and 3.2.4

5.2.3 Physicochemical properties

Described in section 3.2.6; 3.2.7; 3.2.10; 3.2.11; and 3.2.12

5.2.4 Cooking quality

Described in section 3.2.18.

5.2.5 Textural characteristics

Described in section 3.2.18.

5.2.6 Colour measurement

Described in section 3.2.17.

5.2.7 *In vitro* starch digestibility

Describe in section 3.2.20.

5.2.8 Statistical analysis

Described in section 3.2.24 and 3.2.24.1.

5.3 Result & discussion

5.3.1 Effect of cassava and banana flours blend on physico-chemical characteristics of gluten-free pasta

The physico-chemical properties of raw material and gluten-free pasta obtained from cassava flour and banana flour was compared to the control (Table 5.1). The moisture contents of the gluten-free pasta were generally higher than the control. This can be caused by a greater moisture content of initial flours (Table 5.1). Total starch content of the gluten-free pasta containing cassava flours was higher than pure banana pasta and the control pasta. It also can be seen that the gluten-free pasta had a greater ash content than the control. These results were similar to those obtained by Zandonadi et al. (2012) and Baah et al. (2005) which found that banana pasta and cassava pasta have a higher moisture and carbohydrate content then semolina pasta. All gluten-free pasta had significantly lower protein content compared to the control. The differences of physico-chemicals characteristics of gluten-free pasta appears to have positive correlated with initial content of both banana and cassava flours compared to semolina flour. This result was different from other gluten-free pasta experiments using banana or cassava flour (Baah et al., 2005; Fiorda et al., 2013a; Leonel et al., 2011; Sarawong et al., 2014a), since they made some improvements in their pasta making, either by still using wheat ingredient or by protein addition.

Table 5.1 Physio-chemical properties of raw materials and gluten-free pasta compared to semolina pasta (% dry basis)

Type of flour/pasta	Moisture	Total starch	Protein	Ash
Flour				
Semolina	14.88 ± 0.02^{a}	77.20 ± 2.25^{b}	12.36 ± 0.03^{a}	0.49 ± 0.03^{c}
Cassava	10.84 ± 0.06^{b}	82.30 ± 1.82^{b}	1.41 ± 0.03^{c}	0.85 ± 0.03^{b}
Banana	7.84 ± 0.08^{c}	73.36 ± 0.57^{a}	4.54 ± 0.03^{b}	3.51 ± 0.01^{a}
Pasta				
100% Semolina	67.41 ± 1.40^{d}	72.54 ± 1.92^{d}	12.26 ± 0.09^{a}	0.34 ± 0.02^{f}
100% Cassava	66.73 ± 1.03^{d}	82.10 ± 0.60^{a}	1.13 ± 0.03^{f}	0.70 ± 0.07^{e}
75% Cassava: 25% Banana	71.42 ± 1.03^{bc}	80.51 ± 0.92^{ab}	1.79 ± 0.05^{e}	1.05 ± 0.03^{d}
50% Cassava: 50% Banana	70.47 ± 0.95^{cd}	77.94 ± 0.71^{bc}	2.54 ± 0.03^{d}	1.42 ± 0.02^{c}
25% Cassava: 75% Banana	74.63 ± 0.39^{ab}	77.53 ± 0.64^{c}	3.19 ± 0.06^{c}	1.78 ± 0.02^{b}
100% Banana flour	76.86 ± 1.42^{a}	71.56 ± 0.42^{d}	3.88 ± 0.04^{b}	2.08 ± 0.00^{a}

Mean ± standard deviation. Values within a column followed by the same superscript letter in the same type are not significantly different from each other ($p > 0.05$).

All of the gluten-free flour mixtures gave much higher TDF and IDF (Table 5.2) compared to semolina pasta. The SDF content was also higher in most of the gluten-free pasta formulations. These can be explained by a higher initial fibre content on both cassava and banana flours (Table 5.2). The dietary fibre content in all gluten-free pasta was much higher compared to other researchers who used 100 % banana flour, more than 50 % cassava flour or even whole rice flour-based pasta (Baah et al., 2005; Wang et al., 2018; Zandonadi et al., 2012). Thus, it can be useful to develop nutritious gluten-free pasta since which is enriched with fibre content on gluten-free pasta by adding an additional source of dietary fibre such as rice bran fibre (Gao et al., 2018; Wang et al., 2018).

Table 5.2 Insoluble, soluble, and total dietary fibre of raw material and gluten-free pasta compared to semolina pasta (% dry basis)

Type of Flour/Pasta	IDF	SDF	TDF
Flour			
Semolina	5.42 ± 0.01^{c}	1.65 ± 0.43^{b}	7.07 ± 0.44^{c}
Cassava	9.49 ± 0.19^{b}	1.51 ± 0.29^{b}	10.99 ± 0.11^{b}
Banana	12.91 ± 0.35^{a}	3.55 ± 0.16^{a}	16.46 ± 0.19^{c}
Pasta			
100% Semolina	6.11 ± 0.24^{e}	1.69 ± 0.12^{de}	7.80 ± 0.36^{f}
100% Cassava	8.62 ± 0.24^{d}	1.45 ± 0.12^{e}	10.07 ± 0.36^{e}
75% Cassava: 25% Banana	10.23 ± 0.03^{c}	1.84 ± 0.01^{d}	12.07 ± 0.02^{d}
50% Cassava: 50% Banana	11.65 ± 0.31^{b}	2.39 ± 0.09^{c}	14.04 ± 0.22^{c}
25% Cassava: 75% Banana	12.79 ± 0.52^{b}	2.80 ± 0.02^{b}	15.59 ± 0.50^{b}
100% Banana flour	14.49 ± 0.33^{a}	3.45 ± 0.13^{a}	17.94 ± 0.46^{a}

Mean ± standard deviation. Values within a column followed by the same superscript letter in the same type are not significantly different from each other ($p > 0.05$).

Table 5.3 and 5.4 show the L^*, a^*, and b^* values of pasta before and after cooking. The raw gluten-free pasta from 100 % Cassava showed different lightness (L^*) and redness (a^*) values compared to semolina pasta. However once cooked, the differences were not observed. The other formulations tended to have lower lightness, but higher redness value compare with control even after cooking. A higher proportion of banana flour led to a darker colour of gluten-free pasta which similar with previous research of green banana flour addition on rice pasta (Sarawong et al., 2014a).

Table 5.3 Colour of raw pasta

Pasta Formulation	L^*	a^*	b^*
100% Semolina	94.13 ± 0.28^a	-9.65 ± 0.32^d	29.52 ± 0.31^b
100% Cassava	96.27 ± 0.42^b	-11.53 ± 0.37^e	30.34 ± 0.05^{ab}
75% Cassava : 25% Banana	90.65 ± 0.30^c	-8.31 ± 0.31^c	31.24 ± 0.59
50% Cassava : 50% Banana	87.01 ±0.46^d	-7.00 ± 0.20^b	30.20 ± 0.53^{ab}
25% Cassava: 75% Banana	86.33 ± 0.63^d	-6.02 ± 0.53^b	31.64 ± 1.07^a
100% Banana	82.79 ± 0.56^e	-4.21 ± 0.71^a	31.41 ± 0.69^a

Mean ± standard deviation. Values within a column followed by the same superscript letter are not significantly different from each other (p > 0.05

Table 5.4 Colour of cooked pasta

Pasta Formulation	L^*	a^*	b^*
100% Semolina	88.75 ± 0.22^a	-11.29 ± 0.24^c	28.03 ± 0.70^{bc}
100% Cassava	88.16 ± 0.57^a	-9.74 ± 0.11^c	30.15 ± 0.58^a
75% Cassava : 25% Banana	77.96 ± 0.30^b	-5.00 ± 0.30^{ab}	28.76 ± 0.12^{ab}
50% Cassava : 50% Banana	78.50 ± 0.61^b	-4.48 ± 1.68^a	27.76 ± 1.40^{bc}
25% Cassava : 75% Banana	78.21 ± 0.30^b	-6.32 ± 0.76^{ab}	26.91 ± 0.56^{bc}
100% Banana	78.42 ± 0.10^b	-6.67 ± 0.26^b	26.39 ± 0.19^c

Mean ± standard deviation. Values within a column followed by the same superscript letter are not significantly different from each other (p > 0.05)

5.3.2 Effect of cassava and banana flours blend on cooking quality of gluten-free pasta

The cooking quality of gluten-free pasta is shown in Table 5.5. The gluten-free pasta exhibited lower optimum cooking time (OCT), WAI (water adsorption index) and higher cooking loss (CL) values compared to the control. In addition, most of gluten-free pasta showed similar SI values compared to the control, while bigger composition of banana flour lead to higher SI value than semolina pasta. This observation can be explained by a presence of gluten in the control as it has an important role in making a strong and elastic dough. Furthermore, the protein content of all gluten-free pasta was very low thus suitable a binding network with the starch could not be created (Padalino et al., 2016). The OCTs of the gluten-free pasta were shorter compared to control pasta. This can be caused by the lower protein content. Fiorda et al. (2013a) noted that pasta dough with a higher fibre and protein content absorbed more cooking water and led to less water being available for starch gelatinization, resulting in longer cooking times. This can also be seen in the gluten-free pasta formulation, where the increase of protein and fibre content in gluten-free pasta resulted in a longer OCT. Gluten-free pasta made from 100 % Banana has the lowest OCT, however this could be caused by different water temperature added to the dough (100°C) as this may lead to a partial pre-gelatinization of the banana flour. Fiorda et al. (2013a) reported gluten-free pasta with 10 % pre-gelatinized cassava flour had the shortest OCT compared with wheat pasta and whole wheat pasta (3.0; 5.2 and 7.2 minutes respectively).

Table 5.5 Cooking quality of gluten-free pasta compared to semolina pasta

Pasta Formulation	OCT (mins)	SI (g/g)	WAI (%)	CL (%)
100% Semolina	$7 .0 \pm 0.2^{a}$	2.06 ± 0.05^{cd}	100.92 ± 0.77^{a}	3.70 ± 0.09^{e}
100% Cassava	3.0 ± 0.0^{cd}	2.01 ± 0.20^{d}	23.28 ± 0.91^{e}	24.20 ± 0.08^{b}
75% Cassava : 25% Banana	3.2 ± 0.3^{cd}	2.50 ± 0.12^{bc}	28.63 ± 2.00^{e}	28.75 ± 0.22^{a}
50% Cassava : 50% Banana	3.5 ± 0.0^{c}	2.39 ± 0.11^{cd}	39.17 ± 1.86^{d}	26.27 ± 0.58^{b}
25% Cassava : 75% Banana	4.2 ± 0.3^{b}	2.94 ± 0.06^{ab}	60.74 ± 2.81^{c}	21.96 ± 0.87^{c}
100% Banana	2.8 ± 0.3^{d}	3.33 ± 0.27^{a}	86.81 ± 2.59^{b}	15.18 ± 1.59^{d}

Mean ± standard deviation. Values within a column followed by the same superscript letter are not significantly different from each other ($p > 0.05$).

5.3.3 Effect of cassava and banana flours blend on textural characteristic of gluten-free pasta

Firmness and tension of gluten-free pasta compared to semolina pasta as a control are shown in Table 5.6. All the gluten-free pasta formulations have lower firmness and tension compared to semolina pasta, which illustrated a poor texture quality of all experimental treatments. Similar results have been recorded by Zandonadi et al. (2012) and Sarawong et al. (2014a) who noted that the increase of rice flour replacement with banana flour in gluten-free pasta formulation led to a decreasing of firmness. This indicates that the protein content of banana flour has a lower functional quality compared to wheat and the amount of protein on the blend was not sufficient to promote a good textural quality of gluten-free pasta (Sarawong et al., 2014a); (Zandonadi et al., 2012).

Table 5.6 Firmness and tension of gluten-free pasta compared to semolina pasta

Pasta Formulation	Firmness (g)	Tension (g)
100% Semolina	130.54 ± 5.14[a]	34.45 ± 1.55[a]
100% Cassava	51.46 ± 6.39[d]	11.21 ± 0.89[d]
75% Cassava : 25% Banana	58.78 ± 4.31[c]	12.34 ± 0.70[c]
50% Cassava : 50% Banana	56.45 ± 3.24[cd]	13.14 ± 1.37[cd]
25% Cassava: 75% Banana	61.90 ± 4.18[c]	12.42 ± 1.51[c]
100% Banana	71.95 ± 3.93[b]	14.37 ± 3.64[b]

Mean ± standard deviation. Values within a column followed by the same superscript letter are not significantly different from each other ($p > 0.05$).

5.3.4 Effect of different formulation of cassava and banana pasta compared to semolina pasta on the glycaemic properties

Figure 5.1 shows the effect of the different formulations of the cassava and banana flours in gluten-free pasta on reducing sugars released compared to semolina pasta. A higher proportion of cassava flour gave higher values of reducing sugars released during in vitro digestion. On the contrary, the use of banana flour in gluten-free pasta dough reduced the reducing sugars released, even when compared with conventional pasta made from semolina flour. The higher reducing sugars released by cassava flour can be explained by lack of protein content in cassava pasta compare to banana and semolina pasta. Furthermore, a higher dietary fibre content in banana pasta could be the factor that leads to a lower reducing sugar released. These conditions also were found in previous research which showed that sufficient fibre and protein content in the dough can inhibit enzyme to degrade the starch (Bustos, Perez, and León, 2011; Foschia et al., 2014, 2015b; Laleg, Cassan, Barron, Prabhasankar, and Micard, 2016b).

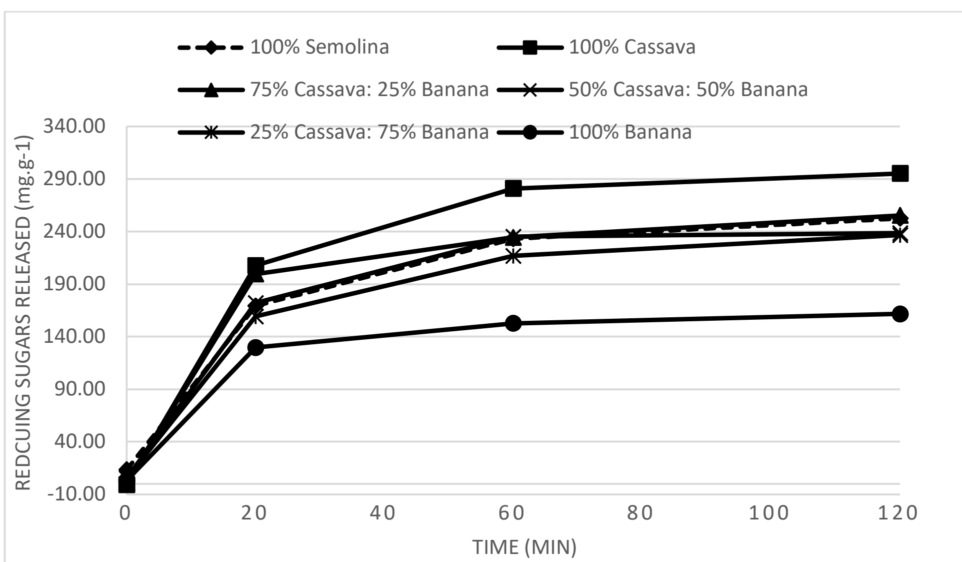

Figure 5.1 Reducing sugars released during in vitro digestion for semolina pasta and gluten-free pasta made from banana and cassava flours

The predictive glycaemic load values of the pastas are the derived from the area under the curve (AUC) and are shown in (figure 5.2). This clearly shows that inclusion of banana flour lowers the AUC and that it occurs in a stepwise fashion with increasing banana flour, such that pasta made with 100 % had an AUC value that is significantly lower than all other pasta. Gluten-free pasta made from 100 % cassava flour had the highest AUC and was significantly different to all the other pasta. The combined formulations did not have an AUC significantly different to the control. Similar results were found by Flores-Silva et al. (2014) who studied gluten-free spaghetti and Agama-Acevedo, Islas-Hernández, Pacheco-Vargas, Osorio-Díaz, and Bello-Pérez (2012) who worked on banana cookies.

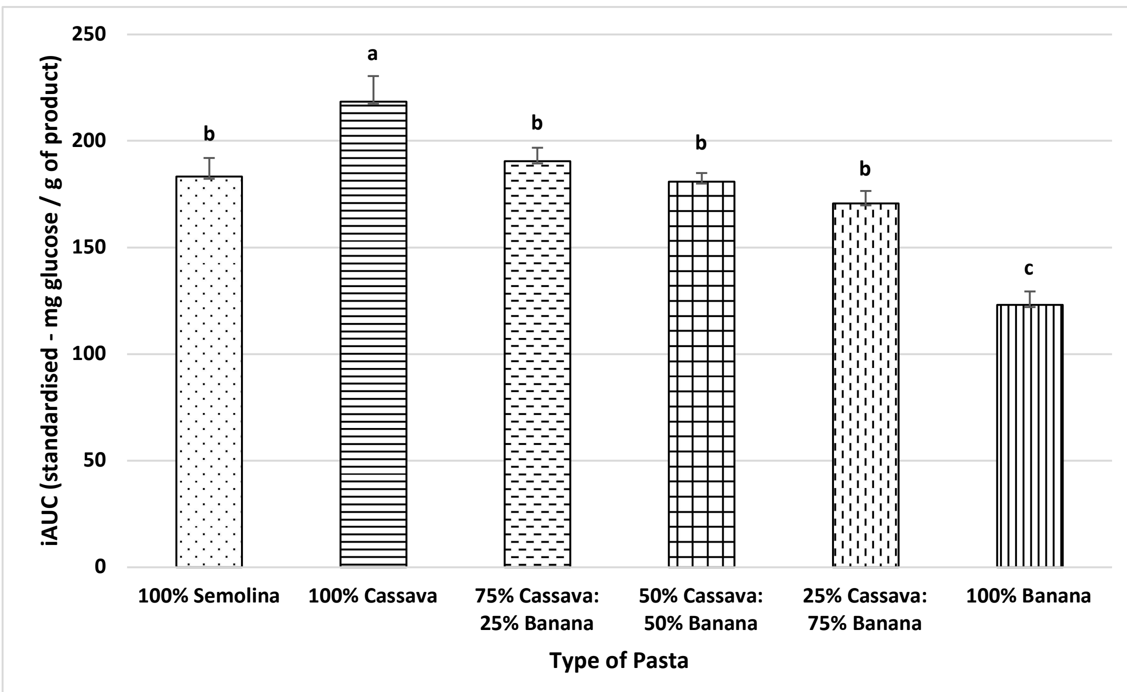

Figure 5.2 Area under curve (iAUC) values of gluten-free pasta made from cassava and banana flours compared to semolina pasta as a control
(different letters above the bars showed significant difference between samples)

Flores-Silva et al. (2014) used up to 30 % unripe plantain combined with 70 % chickpea flour in gluten-free spaghetti and found that the predictive glycaemic index decreased significantly compared to semolina pasta. Agama-Acevedo et al. (2012) used unripe banana flour to substitute wheat flour up to 50 % in cookie formulation and observed a decrease in value of predicted glycaemic index as the amount of unripe banana flour ratios increased in the dough. The increasing fibre content and resistant starch on the blend were suggested to be the cause of the decreasing of AUC values (Agama-Acevedo et al., 2012; Flores-Silva et al., 2014; Foschia et al., 2014).

Studies on cassava flour on gluten-free pasta making especially on its glycaemic properties are still scarce. Some research worked on pasta containing cassava flour did not measure glycaemic properties (Baah et al., 2005; Fiorda et al., 2013a). Baah et al. (2005) only reported the preferred pasta made from composite flour, where the most acceptable ingredients was pasta made from 50% wheat flour and 50% cassava flour, there is not any nutrition nor glycaemic properties provided. (Fiorda et al., 2013a) worked with cassava starch, cassava bagasse and amaranth flour to make gluten-free pasta. They found that the formulation containing 60 % cassava starch, 10% pre-gelatinized flour (made from 70:30 cassava starch and cassava bagasse flour) and 30 % amaranth flour gave a desirable quality and sensory acceptance. Okafor et al. (2017), who worked on bread making found that cassava flour substitution of wheat flour of up to 30 % did not have a significant effect on the glycaemic index of bread. However, it has been debated that most the cassava varieties have high glycaemic properties so its utilization in food products must be selected carefully (Astuti, Hendriyani, and Isnawati, 2013; Oluba et al., 2017). Astuti et al. (2013) studied coconut and black-eyed pea addition into cassava products. It was observed that cassava processing and the addition of coconut and black-eyed pea had significant effects to reduce glycaemic index compared to a conventional steamed cassava product. Oluba et al. (2017) reported on two different varieties of cassava in Nigeria (white gari and yellow gari) and found that the yellow gari variety had a lower glycaemic index.

5.4 Conclusion

The use of cassava and banana flours for gluten-free pasta resulted in higher fibre content compare to semolina pasta. However, the gluten-free pasta made from cassava and banana flours had a lower protein content than durum semolina pasta. In addition, pure cassava pasta did not show different colour with control while other formulation appeared darker (lower lightness and higher redness values). The lack of protein content on cassava and banana pasta decreased the SI, WAI and CL compared to the control pasta made from semolina.

Different proportions of banana and cassava gave significantly different textural quality compare to the control. All gluten-free pasta formulations had weaker firmness and tension values than the control. It was also noted that a higher percentage of banana flour in gluten-free pasta led to better values of firmness and tension. None of the gluten-free pasta produced textural quality similar to the control and these results are an important place to begin future improvement.

The cassava flour and banana flour each had a different effect on the in vitro starch digestibility profile. A higher proportion of cassava flour in the experimental gluten-free pasta showed the higher amount of reducing sugar release compared to control pasta and a higher composition of banana flour led to lower glucose released values. The evaluation of AUC values showed cassava pasta had a higher value compared to the control, while the banana pasta had a lower one.

The results highlight two interesting parts of the utilization of cassava flour and banana flour as alternative ingredients in developing gluten-free pasta. First, the high total dietary fibre of banana and cassava flour can be considered as an important aspect to develop gluten-free pasta with improved nutrition values. Secondly, the utilization of cassava flour and banana flour in gluten-free pasta making would not necessarily have significant effect on predictive glycaemic loading. However, incorporating a higher proportion of banana flour in the pasta formulation leads to lower glycaemic loading properties. Thus, these findings can be used to develop gluten-free pasta from banana flour and cassava flour with some further improvement to achieve better nutritional value with desirable quality to conventional pasta made from durum wheat semolina.

All gluten-free pasta formulations had lower physicochemical and pasta quality compared to the semolina pasta. Hence, the gluten-free pasta needs to be improved by adding either egg white protein or soy protein isolate into the pasta formulation. Based on the results, the best formulations were 100% banana flour formulation, followed by 75% banana and 25% cassava flour formulation. These formulations were used for the next stage of the research.

Chapter 6

The Effects of Egg White Protein and Soy Protein Fortification on Physicochemical Characteristics of Gluten-Free Banana Pasta

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Abstract

Research was conducted to investigate the effects of egg white protein and soy protein powder addition on physico-chemical properties of banana pasta. The levels of protein fortification were 5, 10 and 15% of banana flour (w/w) for each type of protein. Pasta made from 100% durum wheat semolina and 100% banana were used as controls. The addition of soy/egg white protein showed significant differences on physicochemical properties compared to semolina pasta and pure banana pasta. Type of protein, level of protein and the interaction between type of protein and the level of protein altered protein content. Soy protein gave higher protein content than egg white protein, whereas higher level of protein addition gave higher protein content of pasta. The fortified banana pasta had higher protein content than semolina pasta. There were no significant effects of soy/egg white protein addition to either insoluble or soluble dietary fibre content. It was also observed that the cooking properties of pasta (optimum cooking time, swelling index, water adsorption index, and cooking loss) were affected by the level of protein addition and the type of protein. Textural analysis for tension and firmness of the pasta illustrated that protein fortification

improved the textural characteristic of banana flour pasta. The addition of soy or egg white protein also improved the protein content of banana pasta making it equal to or even better then semolina pasta.

Key words: *banana pasta, soy protein, egg white protein, fortification*

6.1 Introduction

Green banana flour or green plantain flour has been successfully used as a replacement or base material in pasta making, either in wheat flour base or gluten-free pasta (Agama-Acevedo et al., 2009a; Choo and Aziz, 2010; Flores-Silva et al., 2014; Ramli, Alkarkhi, Yeoh Shin, Liong, and Easa, 2009; Sarawong et al., 2014a; Zandonadi et al., 2012) Whole unripe banana pasta has been found to have a greater sensory quality compare with the wheat-based pasta (Zandonadi et al., 2012).

The disadvantage of the gluten-free flours in pasta making is they cannot create a sufficiently strong network between their protein and starch to bind the dough and produce the required quality (Djordjević et al., 2018; Han, Ma, Li, Zheng, and Wang, 2018b; Martínez, Marín, Gili, Penci, and Ribotta, 2017; Srikanlaya, Therdthai, Ritthiruangdej, and Zhou, 2018; Tan, Tan, and Easa, 2018; Wang et al., 2018). Hence, it is necessary to improve the process or develop a new formulation with additive ingredients in order to create adequate sensory and cooking qualities (Padalino et al., 2016).

Some efforts have been made to improve the utilisation of gluten-free materials in pasta making through raw material pre-treatment, processing technology or technical addition to replace gluten functionality (Bouasla et al., 2016; Detchewa et al., 2016; Ferreira et al., 2016;

Fiorda et al., 2013a; Marti et al., 2010; Sarawong et al., 2014a). The use of additives (hydrocolloids (for example gums or carboxyl methyl cellulose) and emulsifiers) has been shown to improve the quality and sensory properties of gluten-free pasta (Hager et al., 2012). However, it was discovered that these applications lead to consumers perceiving the product as “artificial food” (Marti et al., 2013). Thus, it was not surprising that the most frequent technical addition in commercial gluten-free products is egg powder (Hager et al., 2012).

As protein contributes to nutritional values of the product, the challenge is to choose an additive which will give added value to the final gluten-free pasta product. In trying to accommodate healthy lifestyle choices, the challenge is achieved by using natural protein resources such as egg powder or soy protein. The use of 15% egg white protein in gluten-free bread making has led to better quality. This proportion also improves the quality of bread in the presence of hydroxypropyl methylcellulose as another additive (Crockett, Ie, and Vodovotz, 2011). Furthermore, the application of 15% soy protein concentrate not only improved cooking quality but also increased protein content of potato pasta to five times higher than control from rice-based gluten-free pasta (Limroongreungrat and Huang, 2007).

Zandonadi et al. (2012) used a high amount of egg white (31.5%), and incorporated hydrocolloids (2.5% guar gum and 2.5% xanthan gum) to make desirable banana pasta, compared to semolina pasta which was used as a control. Other research using green banana flour as an ingredient showed that 30% rice flour replacement with green banana flour gave the best result (best GF pasta quality) (Sarawong et al., 2014a). Only 6% egg albumen was used in this pasta formulation, but it implemented pre-treatment on rice flour (pregelatinized rice flour). There was no nutritional value noted in the research.

There are no studies to the authors' knowledge, which has tried to develop banana pasta with protein addition alone such as soy protein isolate or egg white protein powder. Hence, the objective of this study was to investigate the effects of the protein type (egg white protein and soy protein) and the level of protein fortification on physicochemical properties (protein, moisture and fibre content, cooking properties, and textural properties) of banana pasta.

6.2 Materials & methods

6.2.1 Raw material

Described in section 3.1.

6.2.2 Raw pasta preparation

Described in section 3.2.2. and 3.2.5

6.2.3 Physicochemical properties

Described in section 3.2.6., 3.2.10., and 3.2.13.

6.2.4 Cooking quality

Described in section 3.2.18.

6.2.5 Textural characteristics

Described in section 3.2.19.

6.2.6 Statistical analysis

Described in section 3.2.24.

6.3 Results & discussion

6.3.1 Physicochemical properties

The moisture content and protein content of cooked pasta are shown in Table 6.1. Banana pasta had both higher moisture and protein content. The higher moisture content may be as a result of greater water addition in the banana pasta formulation and because of an increased rate of absorption during the manufacturing process (Choo and Aziz, 2010). The moisture content of protein fortified pasta showed a decreasing trend with greater protein addition. This can be explained by the carbohydrate-protein interaction between polysaccharides in banana flour with egg/soy protein addition. Desai, Brennan, and Brennan (2018b) found a similar result, the moisture content of fish powder enriched pasta decreased with the increasing of fish powder level. This could be attributed to the greater interaction of polysaccharides-protein in the pasta enriched with protein.

The protein content of fortified banana pasta was significantly different to semolina pasta and banana pasta, without any protein addition, that were used as controls. All protein contents were significantly different to each other. Protein content increased with increasing level of protein addition, with soy protein isolate giving higher protein contents than egg protein powder. The protein fortification increased protein content of banana pasta significantly, and at the level of 15% gave higher protein content than semolina pasta. Previous research done by Zandonadi et al. (2012), who made banana pasta with 47.0% banana flour and 31.5 % egg white resulted in lower protein content (9.30%) compare to semolina pasta. Thus, the use of egg white protein powder or soy protein isolate at 15% provided more protein in banana pasta compared to conventional pasta made from semolina (Table 6.1).

Table 6.1 Moisture and protein content of raw materials and cooked pasta (% dry basis)

Sample	**Moisture**	**Protein**
Semolina flour	14.88 ± 0.02	12.36 ± 0.03
Banana flour	7.84 ± 0.08	4.54 ± 0.03
Semolina pasta	67.41 ± 1.40^{e}	12.26 ± 0.09^{c}
Banana pasta	76.85 ± 1.42^{ab}	3.88 ± 0.04^{h}
BE5	78.64 ± 1.54^{a}	7.49 ± 0.05^{g}
BS5	71.16 ± 1.56^{cde}	8.06 ± 0.03^{f}
BE10	74.92 ± 0.61^{abc}	10.83 ± 0.07^{e}
BS10	70.79 ± 0.25^{de}	11.39 ± 0.02^{d}
BE15	73.40 ± 1.57^{bcd}	14.36 ± 0.14^{a}
BS15	69.55 ± 1.83^{de}	13.87 ± 0.02^{b}
General linear model with semolina pasta excluded		
Type of protein powders		
Egg white protein	75.96^{a}	9.14^{b}
Soy protein isolate	72.09^{b}	9.30^{a}
Level of protein powders		
0%	76.86^{a}	3.88^{d}
5%	74.92^{ab}	7.77^{c}
10%	72.86^{bc}	11.11^{b}
15%	71.47^{c}	14.12^{b}

BE5, BE10, BE15: Pasta prepared from 100% banana flour with 5, 10, 15 g addition of egg white protein powder/100 g flour. BS5, BS10, BS15: Pasta prepared from 100% banana flour with 5, 10, 15 g addition of soy protein isolate/100 g flour. Results in the table represent the mean of triplicate measurements. Mean ± standard deviation. Values within a column within a group followed by the same superscript letter are not significantly different from each other ($p > 0.05$), according to Tukey's test.

Soluble dietary fibre (SDF), insoluble dietary fibre (IDF) and total dietary fibre (TDF) are shown in Fig. 6.1. Banana flour contained more dietary fibre than semolina flour, this resulted in a higher fibre content in banana pasta. The addition of 15 % egg or soy protein did not have a significant effect on the dietary fibre content of banana pasta. Most of the dietary fibre in banana pasta is insoluble. These results are similar to previous research utilising banana flour as a noodle ingredient (Choo and Aziz, 2010; Sarawong et al., 2014b). Choo and Aziz (2010) incorporated 30% banana flour into wheat noodles and found that the insoluble dietary fibre content increased compared to the control noodle. The insoluble dietary fibre content is attributed to the hemicellulose content of banana flour and benefits intestinal health by reducing digestion transit time and protecting against colon cancer (da Mota et al., 2000).

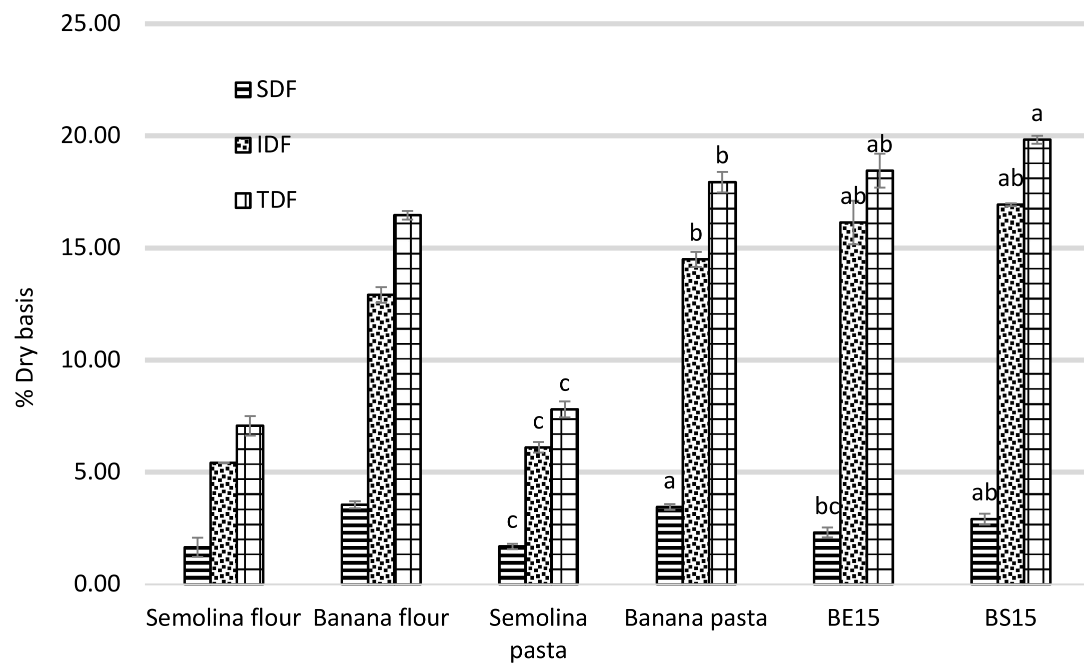

Figure 6.1 Insoluble dietary fibre (IDF), soluble dietary fibre (SDF) and total dietary fibre (TDF) content for raw materials, semolina pasta (control), banana pasta and banana pasta with protein addition

(BE15 = Banana pasta with 15 g egg white protein/ 100 g banana flour, BS15 = Banana pasta with 15 g soy protein/ 100 g banana flour). Bars in the same colour with same letter are not significantly different from each other (P>0.05), according Tukey's test.

6.3.2 Cooking properties

Cooking properties and physical characteristics were analysed by measuring optimum cooking time (OCT), swelling index (SI), water absorption index (WAI) and cooking loss (CL). Cooking properties of cooked pasta are shown in Table 6.2. Research using banana flour as the main or a partial ingredient has shown that the cooking quality of pasta decreased with an increasing proportion of banana flour (Flores-Silva et al., 2014; Sarawong et al., 2014a; Zandonadi et al., 2012). Efforts to improve the quality of gluten-free pasta with protein addition (egg and soy) has proven to be successful (Limroongreungrat and Huang, 2007; Zandonadi et al., 2012).

Cooking loss is the most popular variable to determine the quality of pasta and has become an industry standard. The industry standard of cooking loss for the pasta is no greater than 8 % (Foschia et al., 2015a). Banana pasta with the addition of 10% or 15% protein meets the required standard for cooking loss. The cooking loss of banana pasta was significantly affected by the level and type of protein fortification. Increasing the level of protein inclusion resulted in a decrease of cooking loss, with egg protein giving a lower value than soy protein. Marti et al. (2013) reported that egg albumen was found to be much more efficient than whey protein to lower the cooking loss of gluten-free pasta based on rice flour. Limroongreungrat and Huang (2007) reported different results from the addition of soy protein into potato pasta. They found an increased cooking loss was observed by the increasing level of soy protein concentrate. This could be because the addition of protein was quite high, from 15 g to 45 g/ 100 g potato flour. In this case, the addition of 15% of soy protein into the gluten-free pasta formulation might be the optimum condition to improve gluten-free pasta quality.

All banana pasta had a lower optimum cooking time (2.7-5.0 min) compared to semolina pasta (7.0 min) and this is in agreement with other gluten-free pasta research (Phongthai et al., 2017; Rosa-Sibakov et al., 2016; Sarawong et al., 2014a; Susanna and Prabhasankar, 2013). Phongthai et al. (2017) incorporated various types of protein (egg albumen, rice bran protein concentrate, whey protein, and soy protein concentrate) into rice pasta at rates of up to 9 g/100 g rice flour and observed that the optimum cooking time ranged from 4.25 to 6.48 min. This happened due to poor structure in the absence of a gluten network resulting in faster water penetration into the pasta structure. The shorter optimum cooking time appears to be related to a lower water absorption (81.13 %-97.92% for banana pasta compare with 100.92% for semolina pasta) and higher cooking loss (4.77%-15.18% versus 3.70%). These results were also observed by other authors who found positive correlation between optimum cooking time and water absorption index (de la Peña and Manthey, 2014; Desai et al., 2018b; Petitot, Barron, Morel, and Micard, 2010).

Soy protein did not give significant changes in the WAI, however increasing egg protein increased the WAI. This result indicates the different effects of incorporating animal protein or plant protein into gluten-free pasta. Phongthai et al. (2017) reported similar results with a wide range of animal proteins (egg albumin and whey protein) which also gave higher WAI values compared with plant proteins (rice bran protein concentrate and soy protein concentrate). Wong and Kitts (2003) made comparison of some protein resources and found that soy protein had a poorer protein solubility and total solubility in water compared to dried egg white. Solubility may affect functional properties of protein such as emulsification, gelation, and foam formation (Hettiarachchy, Griffin, and Gnanasambandam, 1996).

Table 6.2 **Cooking properties of cooked pasta**

Type of Pasta	OCT (min)	SI (g/g)	WAI (%)	CL (%)
100% Semolina	7.0 ± 0.2^{a}	2.06 ± 0.05^{d}	100.92 ± 0.77^{a}	3.70 ± 0.09^{f}
100% Banana	2.8 ± 0.3^{d}	3.33 ± 0.27^{ab}	86.81 ± 2.59^{bc}	15.18 ± 1.59^{a}
BE5	4.0 ± 0.0^{c}	3.70 ± 0.35^{a}	87.21 ± 5.00^{bc}	9.71 ± 0.12^{c}
BS5	2.7 ± 0.3^{d}	2.47 ± 0.19^{cd}	83.69 ± 3.94^{bc}	11.67 ± 0.30^{b}
BE10	4.5 ± 0.0^{bc}	2.99 ± 0.10^{abc}	92.06 ± 7.50^{ab}	5.37 ± 0.53^{ef}
BS10	2.8 ± 0.3^{d}	2.42 ± 0.03^{cd}	81.13 ± 1.99^{c}	8.46 ± 0.36^{cd}
BE15	5.0 ± 0.0^{b}	2.77 ± 0.23^{bcd}	97.92 ± 1.53^{a}	4.77 ± 0.10^{ef}
BS15	3.2 ± 0.3^{d}	2.14 ± 0.44^{d}	82.80 ± 1.61^{bc}	6.62 ± 0.67^{de}
General linear model with semolina pasta excluded				
Type of protein powders				
Egg white protein	4.08^{a}	3.20^{a}	97.71^{a}	8.76^{b}
Soy protein isolate	2.88^{b}	2.59^{b}	83.61^{b}	10.48^{a}
Level of protein powders				
0%	2.83^{c}	3.33^{a}	86.82^{b}	15.18^{a}
5%	3.33^{b}	3.09^{ab}	85.45^{b}	10.70^{b}
10%	3.67^{b}	2.71^{bc}	86.59^{b}	6.92^{c}
15%	4.08^{a}	2.46^{c}	103.77^{a}	5.69^{c}

BE5, BE10, BE15: Pasta prepared from 100% banana flour with 5, 10, 15 g addition of egg white protein powder/100 g flour. BS5, BS10, BS15: Pasta prepared from 100% banana flour with 5, 10, 15 g addition of soy protein isolate/100 g flour. Results in the table represent the mean of triplicate measurements. Mean ± standard deviation. Values within a column within a group followed by the same superscript letter are not significantly different from each other ($p > 0.05$), according to Tukey's test.

6.3.3 Textural properties

The firmness and tension of cooked pasta are shown in Table 6.3. Type of protein and rate of inclusion gave significant different firmness values compared to semolina pasta and pure banana pasta. Firmness increased with increasing protein fortification in the banana pasta. Egg protein gave higher firmness values than the soy protein which agrees with previous research regarding the egg and soy protein incorporation into pasta (Larrosa et al., 2016; Marti and Pagani, 2013; Phongthai et al., 2017; Sarawong et al., 2014a; Zandonadi et al., 2012). Egg protein powder had a superior function compared to other protein sources due to its compact structure and denaturation during cooking improved the firmness (Alamprese et al., 2007; Phongthai et al., 2017).

Table 6.3 Firmness and tension of cooked pasta

Type of pasta	Firmness (g)	Extensibility (g)
Semolina	130.54 ± 5.14^{e}	34.45 ± 1.55^{a}
100% Banana	71.95 ± 3.93^{f}	14.37 ± 3.64^{c}
BE5	147.17 ± 8.02^{d}	25.00 ± 3.19^{b}
BS5	150.04 ± 4.23^{d}	17.86 ± 2.36^{c}
BE10	214.74 ± 7.50^{b}	24.33 ± 2.65^{b}
BS10	173.40 ± 5.94^{c}	23.30 ± 2.63^{b}
BE15	265.86 ± 6.14^{a}	33.68 ± 1.97^{a}
BS15	173.25 ± 4.41^{c}	25.77 ± 2.12^{b}
General linear model with semolina pasta excluded		
Type of protein powders		
Egg white protein	174.93^{a}	24.34^{a}
Soy protein isolate	142.16^{b}	20.32^{b}
Level of protein powders		
0%	71.95^{b}	14.37^{d}
5%	148.61^{c}	21.42^{c}
10%	194.07^{b}	23.82^{b}
15%	219.55^{a}	29.72^{a}

BE5, BE10, BE15: Pasta prepared from 100% banana flour with 5, 10, 15 g addition of egg white protein powder/100 g flour. BS5, BS10, BS15: Pasta prepared from 100% banana flour with 5, 10, 15 g addition of soy protein isolate/100 g flour. Results in the table represent the mean of triplicate measurements. Mean ± standard deviation. Values within a column within a group followed by the same superscript letter are not significantly different from each other ($p > 0.05$), according to Tukey's test.

The extensibility of pasta was also affected by the level and type of protein enrichment in banana pasta. Adequate protein content in the pasta dough creates a polypeptide-protein network which can bind and entrapped starch granule to make strong pasta structures (Chillo, Monro, Mishra, and Henry, 2010; Desai et al., 2018b). Both types of protein enhanced the extensibility value, but egg white protein gave better results at every level of addition and reached comparable quality with semolina pasta at 15% level of fortification. Hence, the level and the type of protein addition in gluten-free pasta, especially for banana pasta with 15% egg white protein, which had the highest similarity to semolina pasta, and should be considered as having great potential to be further developed.

6.4 Conclusion

The study illustrates that the fortification with egg white protein and soy protein has successfully improved the physicochemical characteristics of banana pasta. Fortified banana pasta had enhanced protein fortified content compared with both unfortified banana pasta and semolina pasta and enhanced fibre content compared with semolina pasta. The addition of egg white protein and soy protein also positively affected the cooking quality and texture properties of banana pasta. Optimum cooking time and cooking loss both decreased with the increasing level of protein enrichment. Furthermore, the cooking loss of protein fortified banana pasta met the desirable standard of commercial pasta. It was also observed that the egg white protein addition to banana pasta gave better textural properties than soy protein addition and was comparable with commercial pasta based on durum semolina. The optimum protein addition was 15% for both types of protein since it gave the best physicochemical, cooking quality and textural properties among other gluten-free banana pasta formulations.

Chapter 7

Effect of Egg White Protein and Soy Protein Fortification on Physicochemical Characteristics of Gluten-Free Pasta Made of Cassava-Banana Composite Flour

This chapter is published as:

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Abstract

Egg white protein, and soy protein, were incorporated into a banana and cassava flour blend (75:25) to produce gluten-free pasta. The objectives of study were to investigate the effects of the different protein sources on the physico-chemical properties of gluten-free pasta. The levels of protein inclusion were 5, 10 and 15% of composite flour (w/w) for each type of protein. Pasta made from 100% durum wheat semolina and banana-cassava pasta were used as controls. The protein fortification affected the resistant starch and protein content of gluten-free pasta compared to semolina pasta, and banana-cassava pasta. No significant effects of soy/egg white protein addition were found in either insoluble or soluble dietary fibre content. Cooking properties of pasta (optimum cooking time, swelling index, water adsorption index, and cooking loss) and texture properties (firmness and extensibility) were affected by the level of protein addition and the type of protein. Results showed the utilisation of 25% cassava flour into gluten-free pasta based on banana pasta have a promising application since

it has comparable physico-chemical, cooking properties and texture properties with semolina pasta.

Key words: *banana, cassava, soy protein, egg white protein, gluten-free pasta*

7.1 Introduction

Cassava and banana flours have both been used either in wheat-based or gluten-free pasta formulations with some success. The use of green banana flour in pasta formulation has been researched and shown to be comparable to regular wheat based pasta (Agama-Acevedo et al., 2009a; Choo and Aziz, 2010; Flores-Silva et al., 2014; Ramli et al., 2009; Sarawong et al., 2014a; Zandonadi et al., 2012).

However, the utilisation of cassava flour in composite flours for pasta production, either to replace wheat flour or blended with another gluten-free flour is still limited. Okafor et al. (2017) stated that the level of cassava flour in the composite flour that can be used in food products could be up to 20% due to quality and nutrition concerns. However, cassava starch has been incorporated at up to 30% replacement of wheat flour, with technical addition, and produced comparable noodles (Charles et al., 2007). Higher levels of 50% to 60% cassava flour into either wheat-based pasta or gluten-free pasta formulations also have been achieved with pre-gelatinised cassava flour which improves pasta quality (Baah et al., 2005; Fiorda et al., 2013a; Odey and Lee, 2020).

Protein addition has been applied widely to improve gluten-free products as an option that provides gluten functionality. Egg white protein and soy protein isolate have been used to improve the quality of gluten-free bread. These protein sources have been applied in gluten-free product because of their foam stability alongside with other hydrocolloids application (Crockett et al., 2011). Beyond its nutritional properties, egg white protein has great functional properties for wide use in food hydrocolloids as an binding, coagulation, foaming and emulsifying agent for most of food products including pasta (Yemenicioğlu, Farris, Turkyilmaz, and Gulec, 2020). Srikanlaya et al. (2018) applied soy protein isolate addition to improve the quality of gluten-free rice dough and bread. Limroongreungrat and Huang (2007) added soy protein isolate to improve potato pasta which made it better than rice pasta. Detchewa et al. (2016) also used soy protein isolate to improve spaghetti rice quality, while Sarawong et al. (2014a) and (Zandonadi et al., 2012) incorporated egg white protein to improve the quality of gluten-free pasta based on banana flour. The utilisation of soy protein isolate and egg white protein are not only considered of its good functional and nutritional properties, but also for another critical reason. Soy protein isolate is an option of plant origin protein sources to facilitate vegetarians. Meanwhile, egg white protein is considered as an economical of animal protein source since it is derived as by product of egg yolk application in food industries (Crockett et al., 2011; Masure, Wouters, Fierens, and Delcour, 2019; Zhang et al., 2020).

To the best of the authors knowledge there is no study which used cassava flour with banana flour in the presence of protein source. A previous study has been carried out to evaluate the physico-chemical and digestibility properties of gluten-free pasta made from banana and cassava flours blend. It has been reported that optimum utilisation of cassava pasta was 25% with 75% banana flour, but this concentration still yielded pasta which was weaker in quality compared to semolina pasta (Chapter 5). The effect of the egg white protein and soy protein in gluten-free pasta especially based on banana and cassava flour remain unclear since these protein applications were combined with other treatments such as pre-gelatination starch or another hydrocolloids addition (Sarawong et al., 2014a; Zandonadi et al., 2012). Hence, this study was proposed to evaluate the utilisation of cassava flour and the effects of egg white protein or soy protein isolate inclusion on physico-chemical of gluten-free pasta based on banana flour.

7.2 Materials and methods

7.2.1 Raw material

Described in section 3.1.

7.2.2 Raw pasta preparation

Described in section 3.2.3. and 3.2.5.

7.2.3 Moisture content

Described in section 3.2.6.

7.2.4 Total starch

Described in section 3.2.7.

7.2.5 Resistant starch

Described in section 3.2.9.

7.2.6 Protein content

Described in section 3.2.10

7.2.7 Total dietary fibre

Described in section 3.2.13.

7.2.8 Cooking quality

Described in section 3.2.18.

7.2.9 Textural characteristics

Described in section 3.2.19.

7.2.10 Statistical analysis

Described in section 3.2.24.

7.3 Result & discussion

7.3.1 Physico-chemical properties

Total starch, resistant starch, and protein contents, of the cooked pasta are shown in Table 7.1. Banana-cassava flour had a higher total starch content compared to semolina pasta due to high total starch content in cassava flour. Total starch content decreased with the addition level of protein which altered the chemical composition of gluten-free pasta.

Table 7.1 Total starch, resistant starch, and protein content of raw material and cooked pasta (% dry basis)

Sample	Total starch	Resistant starch	Protein
Semolina flour	77.20 ± 2.25	6.18 ± 0.61	12.36 ± 0.03
Banana flour	73.36 ± 0.57	52.75 ± 0.71	4.54 ± 0.03
Cassava flour	82.30 ± 1.82	1.52 ± 0.14	1.41 ± 0.03
Semolina pasta	72.54 ± 1.92^{b}	4.47 ± 0.17^{f}	12.26 ± 0.09^{c}
Banana-cassava pasta	77.53 ± 0.64^{a}	18.16 ± 0.82^{a}	1.79 ± 0.05^{g}
BCE5	72.63 ± 0.04^{b}	13.54 ± 0.09^{c}	6.81 ± 0.04^{f}
BCS5	69.19 ± 0.16^{c}	13.37 ± 0.28^{c}	6.78 ± 0.04^{f}
BCE10	65.88 ± 0.07^{d}	10.59 ± 0.41^{de}	10.25 ± 0.16^{e}
BCS10	71.17 ± 0.04^{bc}	16.26 ± 0.51^{b}	10.87 ± 0.07^{d}
BCE15	67.07 ± 0.07^{d}	11.80 ± 0.37^{d}	13.56 ± 0.07^{b}
BCS15	63.72 ± 0.10^{e}	9.87 ± 0.38^{e}	14.22 ± 0.09^{a}
General linear model with semolina pasta excluded from the calculation			
Type of protein powders			
Egg white protein	71.31^{a}	13.91^{A}	8.10^{B}
Soy protein isolate	69.87^{b}	14.02^{A}	8.41^{A}
Level of protein powders			
0%	77.53^{a}	18.16^{a}	1.79^{d}
5%	71.90^{b}	14.90^{b}	6.79^{b}
10%	68.13^{c}	12.58^{c}	10.56^{c}
15%	64.80^{d}	10.23^{d}	13.89^{a}

Banana-cassava pasta: Pasta prepared from 75% banana : 25% cassava flours. BCE5, BCE10, BCE15: Pasta prepared from 75% banana : 25% cassava flours with 5,10,15 g addition of egg white protein powder/100 g flour. BCS5, BCS10, BCS15: Pasta prepared from 75% banana : 25% cassava flours with 5,10,15 g addition of soy protein isolate/100 g flour. Results represent the mean of triplicate measurements. Mean ± standard deviation. Values within a column within a group followed by the same superscript letter are not significantly different from each other ($p > 0.05$), according to Tukey's test.

Gluten-free pasta made from banana-cassava composite flour had a higher resistant starch content compared to semolina pasta and fortified gluten-free pasta. This can be explained by the high resistant starch content in banana flour compared to semolina flour (Table 7.1). Cassava flour had lower resistant starch met an agreement with Pereira and Leonel (2014), who studied about resistant starch content in 33 different cassava products including different cassava flours and found resistant starch content in cassava flours ranged from 0.19-2.21% (dry weight). High resistant starch in gluten-free banana flour-based is consistent with previous studies of resistant starch content of pasta or noodles that have incorporated with banana flour (Choo and Aziz, 2010; Ovando-Martinez, Sáyago-Ayerdi, Agama-Acevedo, Goñi, and Bello-Pérez, 2009; Sarawong et al., 2014a; Tiboonbun et al., 2011). Increasing levels of protein fortification resulted in a decreased resistant starch content, following a lower total starch composition in the gluten-free pasta formulation. So far only minimal studies have been carried out into the effect of protein inclusion, in gluten-free pasta, on resistant starch content. Sarawong et al. (2014a) studied the effect of banana flour replacement in rice pasta with egg albumen enrichment and found that at the level of 6% egg albumen enrichment, there was no significant difference on resistant starch content with the increasing banana flour addition 30% to 60% in the gluten-free pasta formulation. Escarpa, González, Morales, and Saura-Calixto (1997) reported decreasing resistant starch formation as a result of bovine serum albumin presence in the potato starch mixture. The starch-protein interaction might not affect on resistant starch formation under gelatinisation and or with combination with retrogradation as a decreasing of resistant starch content (Escarpa et al., 1997). As the optimum cooking time increases with increasing protein addition, this might also lead to change in resistant starch granules which has heat sensitivy resulted on resistant loss during cooking process (Foschia, Beraldo, and Peressini, 2017). Peason correlation analysis of resistant-starch and protein content of gluten-free pasta was -0.960 (P value < 0.05) showed strong relation between these two parameters. Gelencsér, Gál, and Salgó (2010) studied the steps during the production of pasta enriched with resistant starch to find the effect on resistant starch content and discovered that the cooking process significantly reduced the resistant starch content and extrusion process did not alter resistant starch content.

Incorporating protein, as a technical addition to the banana-cassava pasta significantly increased the protein content at all levels. Soy protein isolate gave higher protein contents than egg protein powder. These results are similar to previous research using 100% banana pasta using the same protein fortification. Banana-cassava pasta has a very poor protein content (1.79%) compared to banana pasta as shown in previous work (3.88%) (Chapter 6), however the optimum utilization of cassava flour in gluten-free pasta based on cassava-banana flours blend from the previous study which had the best physochemical characteristics and glycemic properties was 25% cassava and 75% banana flours (Chapter 5).

Results of soluble dietary fibre (SDF), insoluble dietary fibre (IDF) and total dietary fibre (TDF) of raw flours material and pasta samples are shown in figure 7.1. Banana flour and cassava flour contain higher amounts of dietary fibre compared to semolina flour and the banana-cassava pasta, fortified and not fortified, had significantly higher fibre contents than semolina pasta, except for soluble dietary fibre in egg fortified banana-cassava pasta. These results agree with previous research utilising banana flour as a noodle ingredient (Choo and Aziz, 2010; Sarawong et al., 2014a). A previous study (Chapter 6) showed a similar pattern of dietary fibre content in banana pasta and the inclusion of 25% cassava flour in gluten-free pasta with protein fortification did not significantly alter the composition of the dietary fibre.

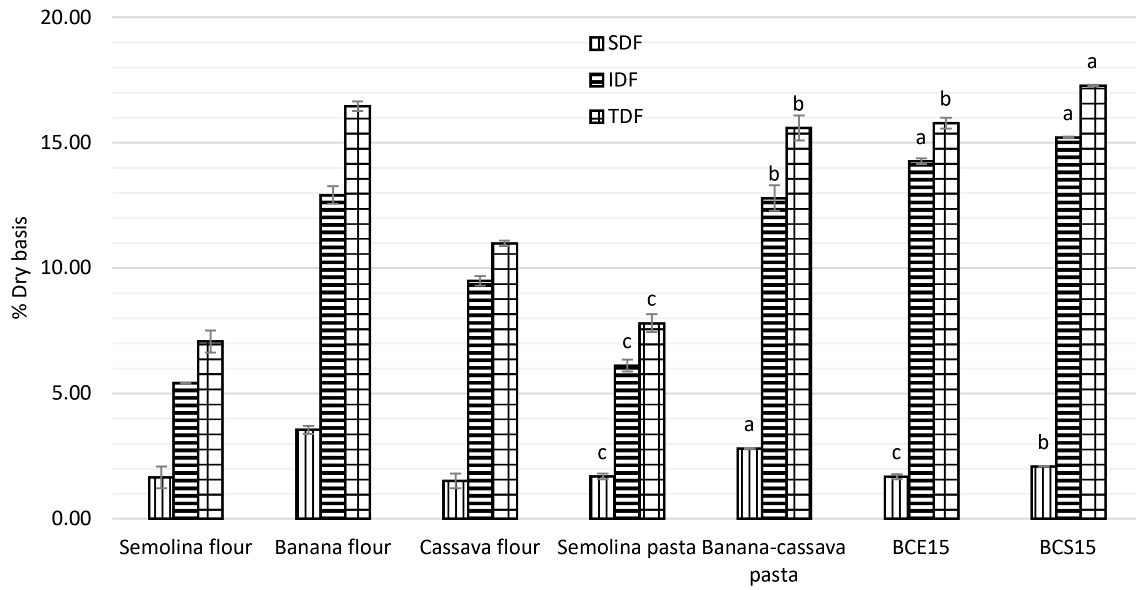


Figure 7.1 Insoluble dietary fibre (IDF), soluble dietary fibre (SDF) and total dietary fibre (TDF) content of raw materials, semolina pasta (control), banana-cassava pasta and banana-cassava pasta with protein addition
(BCE15 = Banana-cassava pasta with 15 g egg white protein/ 100 g banana flour, BCS15 = Banana-cassava pasta with 15 g soy protein/ 100 g banana flour). Bars in the same colour with the same letter are not significantly different from each other (P>0.05), according to Tukey's test.

7.3.2 Cooking properties

The cooking properties of cooked pasta samples are shown on Table 7.2. Previous studies showed that increasing the cassava flour portion, either in wheat-based or gluten-free pasta decreased the cooking quality of the pasta (Baah et al., 2005; Fiorda et al., 2013a). Protein incorporation (egg and soy) into gluten-free pasta has been proven to improve cooking quality to a level comparable with semolina pasta (Limroongreungrat and Huang, 2007; Zandonadi et al., 2012).

Table 7.2 Cooking properties of cooked pasta

Type of Pasta	OCT (mins)	SI (g/g)	WAI (%)	CL (%)
100% Semolina	6.83 ± 0.29^{a}	2.07 ±0.1352^{b}	101.59 ± 0.298^{bc}	3.70 ± 0.09^{d}
Banana-cassava pasta	4.17 ± 0.29^{c}	2.94 ± 0.0597^{a}	60.74 ± 2.81^{e}	21.96 ± 0.87^{a}
BCE5	4.00 ± 0.00^{c}	3.29 ± 0.409^{a}	90.84 ± 3.66^{cd}	7.35 ± 1.08^{c}
BCS5	2.50 ± 0.00^{d}	2.10 ± 0.0495^{b}	75.32 ± 8.87^{de}	10.60 ± 0.63^{b}
BCE10	4.50 ± 0.00^{bc}	2.17 ± 0.199^{b}	110.86 ± 7.05^{ab}	4.11 ± 0.60^{d}
BCS10	2.67 ± 0.29^{d}	2.37 ± 0.0890^{b}	79.29 ± 7.19^{d}	8.84 ± 0.47^{bc}
BCE15	5.00 ± 0.00^{b}	2.19 ± 0.0607^{b}	119.66 ± 6.61^{a}	3.80 ± 0.67^{d}
BCS15	3.00 ± 0.00^{d}	2.12 ± 0.0798^{b}	82.24 ± 1.82^{d}	7.64 ± 0.82^{c}
General linear model with semolina pasta excluded from the calculation				
Type of protein powders				
Egg white protein	4.42^{A}	95.5238^{A}	2.65^{A}	9.31^{B}
Soy protein isolate	3.08^{B}	74.3992^{B}	2.38^{B}	12.26^{A}
Level of protein powders				
0%	4.17^{a}	60.74^{c}	2.94^{a}	21.96^{a}
5%	3.25^{c}	83.08^{b}	2.70^{a}	8.977^{b}
10%	3.58^{b}	95.07^{a}	2.30^{b}	6.48^{c}
15%	4.00^{a}	100.95^{a}	2.15^{b}	5.72^{c}

Banana-cassava pasta: Pasta prepared from 75% banana : 25% cassava flour. BCE5, BCE10, BCE15: Pasta prepared from 75% banana : 25% cassava flours with 5,10,15 g addition of egg white protein powder/100 g flour. BCS5, BCS10, BCS15: Pasta prepared from 75% banana : 25% cassava flours with 5,10,15 g addition of soy protein isolate/100 g flour. Results represent the mean of triplicate measurements. Mean ± standard deviation. Values within a column within a group followed by the same superscript letter are not significantly different from each other ($p > 0.05$), according to Tukey's test.

All of the gluten-free pasta samples had a lower optimum cooking time (2.5-5.0 min) compared to semolina pasta (7.0 min) which is in agreement with previous gluten-free pasta research (Phongthai et al., 2017; Rosa-Sibakov et al., 2016; Sarawong et al., 2014a; Susanna and Prabhasankar, 2013). The absence of a gluten network and a poor pasta structure results in faster water penetration rate and a reduced optimum cooking time in gluten-free pasta compared to semolina pasta (Phongthai et al., 2017). The addition of egg white protein to the pasta increased the optimum cooking time, while the addition of soy protein did not show any significant differences. A previous study using banana flour with egg white protein and soy protein isolate resulted showed a similar trend of changes in optimum cooking time (Chapter 6).

The swelling index of pasta samples can be seen in Table 7.2. Gluten-free pasta control made from banana-cassava composite flour (no protein addition) had a higher swelling index than semolina pasta. Protein addition to the gluten-free pasta formulation decreased the swelling index such that it was not significantly different to the semolina pasta. Similar results were found in other studies regarding protein fortification during pasta making (Desai, Brennan, and Brennan, 2019; Liu et al., 2016). A decreasing swelling index might be the result of the formation of a complex network of protein and starch which in turn limits the water for swelling and gelatinisation of the starch granule (Desai et al., 2019).

Water absorption index (Table 7.2) increased with increasing protein fortification. Similar results were found in other research into gluten-free pasta enriched with protein (Detchewa et al., 2016; Marti et al., 2013; Phongthai et al., 2017). Detchewa et al. (2016) found that the water adsorption index of rice spaghetti increased with the addition 2.5 to 10% soy protein isolate but the values were still lower than the commercial wheat spaghetti used as a control. It was also observed that fortification with egg white protein gave a higher water adsorption index compared to soy protein. This result was also similar to previous research which showed that gluten-free pasta with egg white protein addition was more similar to the semolina control than gluten-free pasta with soy protein isolate addition (Phongthai et al., 2017).

Cassava flour inclusion up to 25% in gluten-free pasta with protein addition showed no significant effect on the swelling index value.

The cooking loss value of protein fortified gluten-free pasta samples ranged from 3.80% to 10.60% and were all significantly different from the gluten-free pasta control (21.96%). This shows that the addition of protein decreased cooking loss to an acceptable level in terms of, which is less than 8% (Desai et al., 2019; Magdalena, 2014). Decreased cooking losses with increased protein addition has also been observed in other studies of protein fortified gluten-free pasta (Larrosa et al., 2016; Sarawong et al., 2014a). Larrosa et al. (2016) made gluten-free pasta using corn starch, cornflour and dried egg white, and found that the cooking loss decreased as the level of egg white increased.

7.3.3 Textural properties

Textural properties can reflect the quality of pasta that is acceptable to the consumer. In this study, textural properties were observed by measuring firmness and extensibility (Table 7.3). Firmness is one of the most critical measurements that is used to evaluate the quality of the dough matrix integration (Schoenlechner et al., 2010). It reflects the bond strength and the integrity of the protein matrix present in the cooked pasta (Larrosa et al., 2016). Banana-cassava pasta had a lower firmness compared to semolina pasta (61.90 g compared to 130.54 g) shows that gluten-free pasta without any protein addition had a weaker dough matrix. The addition of protein altered the firmness and increased with increasing protein inclusion. The increased firmness seems to be related not only to the amount bu also the quality of protein present in the pasta mixture. Sarawong et al. (2014a) concluded that protein of low functional quality might cause a decrease in firmness of the gluten-free pasta made from rice flour and green plantain flour. Bravo-Núñez, Sahagún, Bravo-Núñez, and Gómez (2020) found egg white protein and whey protein enrichment gave better textural properties of gluten-free cake compared to vegetable protein. The combination of total starch and protein content in the pasta formulation may also influence the pasta firmness (Zandonadi et al., 2012).

Table 7.3 Firmness and tension of cooked pasta

Type of pasta	Firmness (g)	Extensibility (g)
Semolina	130.54 ± 5.14^{d}	34.455 ± 1.55^{a}
Banana-cassava	61.90 ± 4.41^{f}	12.422 ± 1.51^{cd}
BCE5	103.438 ± 2.48^{e}	17.326 ± 2.38^{c}
BCS5	62.45 ± 4.37^{f}	9.542 ± 1.05^{d}
BCE10	226.30 ± 5.95^{b}	25.26 ± 4.25^{b}
BCS10	106.04 ± 5.74^{e}	14.862 ± 1.28^{c}
BCE15	242.73 ± 7.17^{a}	34.39 ± 8.57^{a}
BCS15	140.93 ± 3.35^{c}	23.056 ± 1.77^{b}
General linear model with semolina pasta excluded from the calculation		
Type of protein powders		
Egg white protein	158.59^{a}	22.345^{a}
Soy protein isolate	92.83^{b}	14.97^{b}
Level of protein powders		
0%	61.90^{d}	12.42^{c}
5%	82.95^{c}	13.43^{c}
10%	166.17^{b}	20.06^{b}
15%	191.83^{a}	28.72^{a}

BCE5, BCE10, BCE15: Pasta prepared from 75% banana : 25% cassava flour with 5,10,15 g addition of egg white protein powder/100 g flour. BCS5, BCS10, BCS15: Pasta prepared from 75% banana : 25% cassava flours with 5,10,15 g addition of soy protein isolate/100 g flour. Results represent the mean of triplicate measurements. Mean ± standard deviation. Values within a column within a group followed by the same superscript letter are not significantly different from each other ($p > 0.05$), according to Tukey's test.

The extensibility of gluten-free pasta was also influenced by the level and type protein present in the pasta blends. A strong pasta structure, which can be defined by a higher value of extensibility, is formed through starch granule entrapment in the presence of sufficient protein in the food matrix (Chillo et al., 2010). A similar pattern of texture properties was found in a previous study that used only banana flour with protein fortification (Chapter 6). These results led to promising utilisation of cassava flour into gluten-free pasta formulation.

7.4 Conclusion

The study showed that egg white protein or soy protein inclusion both successfully enhanced the physico-chemical characteristics of a gluten-free pasta made from a composite flour consisting of banana and cassava (75:25). Protein fortification increased the protein content, fibre content was maintained and resistant starch was decreased. The addition of egg white protein or soy protein both improved the cooking quality and texture properties of the gluten-free pasta. The cooking loss decreased as the level of protein enrichment increased ultimately meeting the desirable standard of commercial pasta. It was also observed that the utilisation of 25% cassava flour into gluten-free pasta did not alter the physico-chemical characteristics, cooking quality or texture properties. The optimum protein addition was 15% for both types of protein since it gave the best physicochemical properties and pasta quality among other gluten-free pasta formulations.

Chapter 8

Effect of Egg White Protein and Soy Protein Fortification on Nutritional Quality of Gluten-Free Banana Pasta

This chapter is published as:

Rachman, A., A Brennan, M., Morton, J., & Brennan, C. S. (2020). Effect of egg white protein and soy protein isolate addition on nutritional properties and *in vitro* digestibility of gluten-free pasta based on banana flour. *Foods (Basel, Switzerland), 9(5). doi:10.3390/foods9050589*

Abstract

The effects of egg white protein and soy protein isolate addition on the nutritional and digestibility of gluten-free pasta based on banana flour were studied. The level of protein additions (soy protein or egg white protein) were 0, 5, 10 and 15% of banana flour (*w*/*w*). Pasta made from 100% durum wheat semolina was used as a control. Soy protein isolate inclusion into banana pasta increased total phenolic content (TPC) and antioxidant capacities, while egg white protein decreased the TPC and antioxidant capacities with the increasing level of addition. Starch digestibility was affected by the type of protein addition. Egg white protein lowered starch digestibility compared to soy protein isolate. Protein inclusion in banana pasta also altered protein digestibility, amino acid profiles and protein digestibility-corrected amino acid score (PDCAAS). Soy protein isolate increased protein digestibility of gluten-free pasta compared to egg white protein. Protein enrichment gave better amino acid profiles of banana pasta compared to semolina pasta, and egg white protein performed a better PDCAAS compared to soy protein isolate. These results showed that soy protein isolate and egg white

protein addition enhanced nutritional qualities and digestibility properties of gluten-free banana pasta.

Keywords: *banana pasta; soy protein isolate; egg white protein; digestibility; amino acid*

8.1 Introduction

The effort to provide suitable pasta for celiac and gluten intolerance-related patients has led to the production of gluten-free (GF) pasta. There are many varieties of GF pasta made from rice and other GF flours available, but these products often have poor cooking quality and technological difficulties compared to conventional wheat pasta as well as having an inferior nutritional quality especially with regard to minerals and bioactive compounds (Duta et al., 2019; Marti and Pagani, 2013). Commercial GF products also often perceived as unattractive from a consumer perspective as unhealthy products and with lots of artificial ingredients (Rybicka, Doba, and Bińczak, 2019).

One GF flour that has great potential to be developed as a GF pasta ingredient because of its nutritional properties is banana flour. Banana flour is derived from unripe bananas which have a high phenolic content and high antioxidant capacity (Aurore, Parfait, and Fahrasmane, 2009). Banana flour is mainly composed of starch, with a relatively low protein content, but the high level of bioactive ingredient in banana makes it useful as a functional food ingredient. The bioactive compounds in bananas remain high and active after processing into banana flour which makes it a good nutritional source for the food industry (Campuzano et al., 2018).

Banana flour has been applied in either wheat-based or GF pasta manufacture as an alternative base material, improving the pasta and making it comparable with wheat only counterparts (Agama-Acevedo et al., 2009a; Choo and Aziz, 2010; Flores-Silva et al., 2014; Ramli et al., 2009; Sarawong et al., 2014a; Zandonadi et al., 2012). It also has been reported in previous work that banana flour has great potential to be developed as gluten-free pasta due to its physicochemical and digestibility properties (Chapter 5).

Some of the research into banana pasta stated the nutritional values of pasta products, but none investigated how the ingredients affected the nutritional and digestibility properties of the final product. Sarawong et al. (2014a) reported a high content of resistant starch in banana pasta but did not observe any phenolic content or antioxidant activities. Ovando-Martinez et al. (2009) reported phenolic content, antioxidant activities and starch digestibility in pasta made from up to 45% banana flour, but still used semolina as a base ingredient. There is limited research discussing the nutritional aspects of gluten-free pasta based on banana flour.

Most commercial GF pasta use hydrocolloids and emulsifier to improve cooking quality which led to the consumer association with artificial flavour (Gao et al., 2018; Morreale et al., 2019). Another functional additive that has been proven to improve the quality of GF product is protein source, such as egg white protein and soy protein isolate (Gao et al., 2018). Protein enrichment with egg protein has successfully improved the cooking quality and textural properties of banana pasta (Sarawong et al., 2014a; Zandonadi et al., 2012). It has also been reported that soy protein isolate addition enhanced gluten-free rice spaghetti quality (Detchewa et al., 2016). Previous research has been carried out to incorporate soy protein isolate and egg white protein into banana flour and has improved the cooking quality and textural properties of banana pasta (Chapter 6). The aim of this study was to investigate the effects of the protein type (egg white protein and soy protein) and the level of protein addition on nutritional qualities (total phenolic content, antioxidant capacities and amino acid profiles) and digestibility properties (starch digestibility and protein digestibility) of gluten-free pasta based on banana flour.

8.2 Materials and Methods

8.2.1. Materials

Banana flour, semolina flour, egg white protein, and soy protein isolate as described in section 3.1.

8.2.2. Pasta Preparation

Described in section 3.2.2. and 3.2.5

8.2.3. Total phenolic content and antioxidant capacity

Described in section 3.2.15.

8.2.4. *In vitro* starch digestibility

Described in section 3.2.20

8.2.5. Protein content for *in vitro* protein digestibility determination

Described in section 3.2.10.

8.2.6. *In vitro* protein digestibility

Described in section 3.2.21.

8.2.7. Amino acid profiles, amino acid score (AAS) and protein digestibility-corrected amino acid score (PDCAAS)

Described in section 3.2.16 and 3.2.22.

8.2.8. Statistical Analysis

Described in section 3.2.24.

8.3 Results and Discussion

8.3.1 Total phenolic content (TPC)

Total phenolic content (TPC) of raw materials and pasta samples are shown in Table 8.1. Banana flour has higher TPC compared to semolina flour, while soy protein isolate contains more TPC compared to egg white protein. These initial statuses led to higher TPC in banana pasta compared to semolina pasta, while addition of soy protein isolate gave a higher TPC compared to egg white protein enrichment. Choo and Aziz (2010) made noodles incorporating 30% banana flour into wheat-based noodles and found significant increase in value of TPC. Noodles with 30% banana flour had more than triple TPC compared to 100% wheat noodle as a control (28.6 and 90.4 mg GAE/100g respectively).

Table 8.1 Total phenolic content and antioxidant capacities of banana pasta and semolina pasta

Sample	TPC (mg GAE/ 100g DM)	FRAP (mmol Fe^{2+}/100g DM)	ABTS (µmol TE/100g DM)
Raw materials			
Semolina flour	73.80 ± 0.78	0.15 ± 0.02	0.67 ± 0.02
Banana flour	116.45 ± 4.75	1.14 ± 0.00	1.46 ± 0.02
Soy protein isolate	261.26 ± 2.50	1.06 ± 0.00	2.72 ± 0.01
Egg white protein	70.19 ± 1.85	0.49 ± 0.00	0.11 ± 0.01
Pasta samples			
Semolina	55.73 ± 0.73^{e}	0.10 ± 0.00^{g}	0.31 ± 0.02^{g}
Banana	63.37 ± 0.05^{c}	1.05 ± 0.03^{a}	1.04 ± 0.01^{c}
BE5	54.66 ± 2.68^{e}	1.02 ± 0.02^{a}	1.01 ± 0.00^{c}
BS5	63.11 ± 1.19^{c}	0.89 ± 0.02^{c}	0.95 ± 0.01^{d}
BE10	58.47 ± 1.86^{cde}	0.98 ± 0.00^{ab}	0.90 ± 0.01^{e}
BS10	79.78 ± 2.10^{b}	0.98 ± 0.00^{ab}	1.22 ± 0.01^{b}
BE15	60.47 ± 1.77^{cd}	0.94 ± 0.01^{bc}	0.73 ± 0.01^{f}
BS15	86.69 ± 2.86^{a}	1.02 ± 0.01^{a}	1.37 ± 0.02^{a}
General linear model with semolina pasta excluded from the calculation			
Type of protein powders			
Egg white protein	59.24^{b}	0.99^{a}	0.92^{b}
Soy protein isolate	73.24^{a}	0.93^{a}	1.15^{a}
Level of protein powders			
0%	58.89^{d}	1.03^{a}	1.04^{b}
5%	63.37^{c}	0.96^{b}	0.98^{c}
10%	69.13^{b}	0.98^{b}	1.06^{a}
15%	73.58^{a}	0.98^{b}	1.05^{ab}

BE5, BE10, BE15: Pasta prepared from 100% banana flour with 5, 10, 15 g addition of egg white protein powder/100 g flour. BS5, BS10, BS15: Pasta prepared from 100% banana flour with 5, 10, 15 g addition of soy protein isolate/100 g flour. Results in the table represent the mean of triplicate measurements. Mean ± standard deviation. Values within a column within a group followed by the same superscript letter are not significantly different from each other ($p > 0.05$), according to Tukey's test.

High TPC in soy protein isolate also contributed to enhancing TPC of banana pasta. The TPC of banana pasta increased by increasing fortification level of soy protein isolate. Soy protein isolate alongside with other soy products has been reported to contain high total phenolic content ranging from 104.3 to 243.1 mg GAE/100 g (Beatriz Cervejeira and Adelaide Del Pino, 2011). Different effects were found with egg white protein inclusion; the level of egg white protein did not give significantly different values of TPC. There is limited research which discuss the effect of protein addition on total phenolic content in either wheat-based pasta or gluten-free pasta.

8.3.2 Antioxidant capacities

Antioxidant activities including ferric reducing/antioxidant power (FRAP) and ABTS radical scavenging capacity of raw materials and pasta sample can be seen in Table 8.1. Similar to the TPC content, banana flour has higher FRAP and ABTS values compared to semolina pasta which then provided higher antioxidant capacities in banana pasta compared to semolina pasta. The higher ABTS values in banana pasta compared to semolina pasta meet agreement with Ovando-Martinez et al. (2009) who carried out a study on undigestible carbohydrate of semolina pasta supplemented with banana flour. They found that the ABTS value of semolina pasta increased with increasing level of banana flour replacement. Semolina pasta had an ABTS value of 0.55 μmol Trolox equivalent/g and increased up to 0.89 μmol Trolox equivalent/g with 45% banana flour inclusion.

Soy protein isolate addition provided higher FRAP and ABTS values compared to egg white protein. A higher soy protein level gave higher antioxidant capacities especially for ABTS values. On the contrary, higher egg white protein incorporation in banana pasta reduced antioxidant activities. This can be explained by lower FRAP and ABTS values in egg white protein, while soy protein isolate has high value similar to banana flour. Soy protein utilization in many food formulations has been established because of its functional and nutritional properties (Tang and Ma, 2009). Soy protein and egg white protein have also been utilised in GF products (Crockett et al., 2011; Phongthai et al., 2017), but none of the previous studies examined the effect of the protein inclusion on nutritional properties of the GF products.

8.3.3 *In vitro* starch digestibility

The effect of protein incorporation on starch digestibility can be seen in Figure 8.1. Egg white protein fortification gave a lower value of area under curve (iAUC) compared to banana pasta with soy protein isolate addition. Banana pasta with egg white inclusion also has a lower iAUC value compared to unfortified banana pasta; however, this is not significantly different.

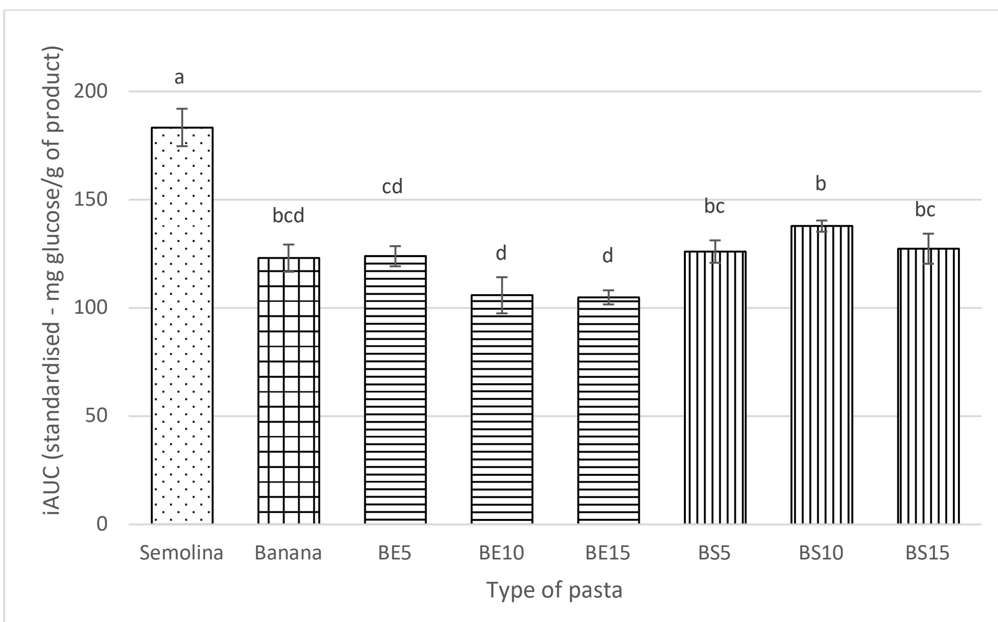


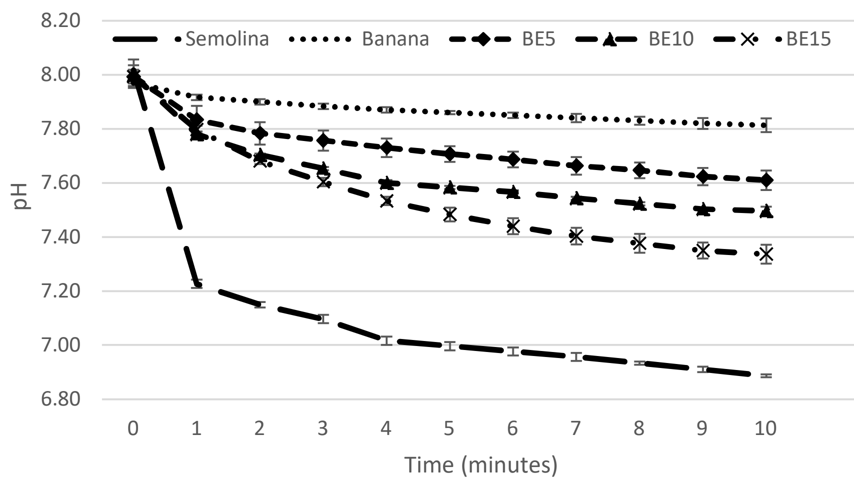
Figure 8.1 Area under curve (iAUC) values of semolina pasta, banana pasta and banana pasta with protein addition
(BE5, BE10, BE15 = Banana pasta with 5, 10, 15 g egg white protein/100 g banana flour, BS5, BS10, BS15 = Banana pasta with 15 g soy protein/100 g banana flour) compared to semolina pasta as a control. Different letters above the bars showed significantly different from each other (*p* > 0.05), according to Tukey's test.

Some research projects have reported that protein inclusion in pasta based on semolina flour arrived at lower *in vitro* starch digestibility properties compared to semolina pasta (Desai et al., 2018; Ramya, Prabhasankar, Gowda, Modi, and Bhaskar, 2015; Rodríguez De Marco, Steffolani, Martínez, and León, 2014). Numerous studies have been conducted to illustrate how starch digestibility is decreased by incorporating more fibre into wheat-based pasta (Brennan, Kuri, and Tudorica, 2004; Bustos et al., 2011; Choo and Aziz, 2010; Foschia et al., 2015b). Unfortunately, there is no published research that addresses the effects of protein inclusion on digestibility properties of gluten-free pasta. Hager et al. (2013) made a comparison of egg pasta made from oat, teff and wheat flours and found that starch digestibility varies according to the pasta composition. Oat based-pasta with a lower egg white powder proportion (9.7%) was reported have a lower glucose released compared to teff based-pasta with higher egg white powder (11.0%). It has also been noted that the protein content of teff flour was higher compared to oat flour (12.8% and 6.9%, respectively), while both flour have similar fibre content (4.5% and 4.1%, respectively). This result explains how a higher protein content in the food matrix may lead to different effect in starch digestibility properties.

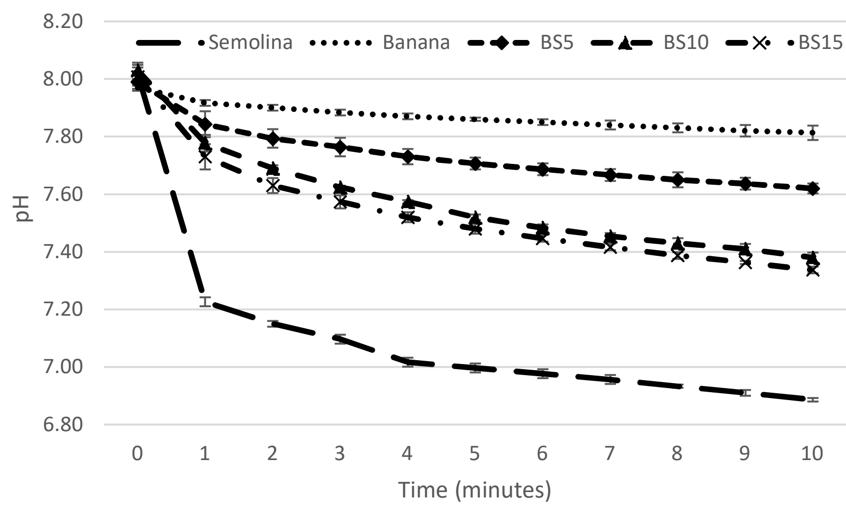
The amount of protein is not the only aspect to consider, but also the quality of protein itself (Hager et al., 2013). Oat protein has a higher lysine content that makes it superior compared to other cereals (Lásztity, 1996), and the differences in protein composition may be why the addition of different types of protein in the same amount may give different effects on starch digestibility. Egg white protein seems to have superior quality compared to soy protein isolate in creating a protein-starch network in banana pasta which alters the starch digestion during the enzymatic reaction. A better quality of protein in the egg white builds a stronger banana pasta structure which can also be illustrated by superior cooking quality and texture properties of enriched banana pasta compared to soy protein isolate inclusion in previous work (Chapter 6). Shen, Tebben, Chen, and Li (2019) studied amino acid enrichment in bread and found the addition of lysine, alanine or glycine improved the textural quality of white bread, implying that protein quality may have an effect on dough structures in food matrices.

8.3.4 *In vitro* protein digestibility

Protein digestion was observed by pH change over time during multi-enzymes (trypsin, chymotrypsin and protease) incubation of pasta samples which can be seen in Figure 8.2. Protein is digested into amino acids and peptides resulting in pH drop. Multi-enzymes break the protein solution into carboxyl ($-COO^-$) and amino ($-NH_3^+$) groups. Free amino groups deionize in water and protons (H^+) are liberated at neutral pH (8.0). The free H^+ released into the solution decreased in pH value. The phenolic present in pasta could react with amino acid creating protein cross-link that inhibits further enzymatic protein degradation (Desai et al., 2018).



(**a**)



(**b**)

Figure 8.2 The pH change over time during multi-enzymes (trypsin, chymotrypsin and protease) incubation of semolina pasta, banana pasta and banana pasta with protein addition

(**a**) BE5, BE10, BE15 = Banana pasta with 5, 10, 15 g egg white protein/100 g banana flour; (**b**) BS5, BS10, BS15 = Banana pasta with 15 g soy protein/100 g banana flour

Protein content, protein digestibility and protein availability of pasta samples are shown in Table 8.2. Banana pasta without protein enrichment has the lowest protein digestibility due to its lack of protein content. Semolina pasta had the highest protein digestibility among all samples because of its high protein content. The effect of protein addition to the banana pasta increased protein digestibility with increasing fortification. Soy protein isolate addition gave higher protein digestibility compared to egg white protein. This result agrees with previous research which illustrated that protein digestibility of some commercial protein supplements including soy protein isolate and egg white protein powder varied greatly. For instance, it has been found that soy protein isolate had higher protein digestibility (89.2%) compared to egg white protein powder (81.5%) (Bustos et al., 2011). Interestingly, enriched banana pasta with similar or higher protein content (11.39–13.89%) compared to semolina pasta (12.26%) had a lower protein digestibility. Simonato, Curioni, and Pasini (2015) observed that protein digestibility of pasta made from three types of wheat flour was not affected by protein content. The lower protein digestibility might be caused by high antinutrient content in banana pasta especially the phenolic content and soluble fibre, both of which can inhibit protein digestion during enzymatic reactions. Anti-nutrient activities against protein digestibility were also suggested by other research into protein digestibility in pasta (Lásztity, 1996; Lim and Murtijaya, 2007b; Tang and Ma, 2009).

Table 8.2 Protein content, protein digestibility and protein availability of cooked semolina pasta and banana pasta

Type of pasta	Protein (% db)	Protein Digestibility (%)	Protein Availability (%)
Semolina	12.26 ± 0.09[c]	85.81 ± 0.10[a]	10.52 ± 0.09[c]
Banana	3.88 ± 0.04[h]	69.04 ± 0.21[e]	2.68 ± 0.04[h]
BE5	7.49 ± 0.05[g]	72.72 ± 0.65[d]	5.44 ± 0.04[g]
BS5	8.06 ± 0.03[f]	72.54 ± 0.31[d]	5.85 ± 0.03[f]
BE10	10.83 ± 0.07[e]	74.77 ± 0.28[c]	8.10 ± 0.08[e]
BS10	11.39 ± 0.02[d]	76.88 ± 0.31[b]	8.76 ± 0.03[d]
BE15	14.36 ± 0.14[a]	77.67 ± 0.64[b]	11.16 ± 0.11[a]
BS15	13.87 ± 0.02[b]	77.67 ± 0.21[b]	10.77 ± 0.02[b]
General linear model with semolina pasta excluded from the calculation			
Type of protein powders			
Egg white protein	9.14[b]	73.55[b]	6.85[b]
Soy protein isolate	9.30[a]	74.03[a]	7.01[a]
Level of protein powders			
0%	3.88[d]	69.04[d]	2.68[d]
5%	7.77[c]	72.63[c]	5.64[c]
10%	11.11[b]	75.83[b]	8.43[b]
15%	14.12[a]	77.67[a]	10.96[a]

BE5, BE10, BE15: Pasta prepared from 100% banana flour with 5, 10, 15 g addition of egg white protein powder/100 g flour. BS5, BS10, BS15: Pasta prepared from 100% banana flour with 5, 10, 15 g addition of soy protein isolate/100 g flour. Results in the table represent the mean of triplicate measurements. Mean ± standard deviation. Values within a column within a group followed by the same superscript letter are not significantly different from each other ($p > 0.05$), according to Tukey's test.

8.3.5 Amino acid profiles and protein digestibility corrected amino acid scores (PDCAAS)

Amino acid profiles (mg per g protein) of the pasta samples can be seen in Table 8.3. The level of protein addition and the type of protein altered amino acid composition in banana pasta. Banana pasta had poor essential amino acid content, e.g., methionine (met), cysteine (cys), and isoleucine (ile), compared to semolina pasta. The addition of egg white protein or soy protein isolate enhanced the amino acid composition to become better balanced for daily requirements than semolina pasta. Egg white protein gave a better ratio of essential amino acid to total amino acid than soy protein isolate inclusion. Table 8.3 shows the comparison of amino acid profiles in the pasta samples with the recommendation of amino acid required for daily adult intake by WHO et al. (2007).

Table 8.3 Comparison of amino acid profiles in semolina pasta and banana pasta with daily adult intake recommendation (mg of amino acid per g protein)

Type of pasta	Amino Acid							
	His	Ile	Leu	Lys	Cys + Met	Phe + Tyr	Thr	Val
Semolina	57 ± 3^{b}	24 ± 1^{d}	53 ± 3^{bc}	46 ± 2^{d}	129 ± 7^{a}	59 ± 3^{c}	33 ± 2^{cd}	29 ± 1^{f}
Banana	137 ± 7^{a}	7 ± 1^{e}	23 ± 1^{d}	127 ± 7^{a}	2 ± 0^{d}	32 ± 1^{e}	64 ± 3^{a}	14 ± 1de
BE5	60 ± 9^{b}	31 ± 4^{bc}	58 ± 4^{bc}	77 ± 12^{b}	71 ± 4^{b}	58 ± 4^{c}	44 ± 6^{b}	40 ± 3^{b}
BE10	53 ± 11^{b}	40 ± 1^{a}	71 ± 0^{a}	78 ± 8^{b}	76 ± 8^{b}	75 ± 1^{ab}	47 ± 4^{b}	51 ± 1^{a}
BE15	40 ± 3^{b}	42 ± 1^{a}	74 ± 3^{a}	73 ± 3^{bc}	73 ± 4^{b}	78 ± 1^{a}	44 ± 2^{b}	52 ± 1^{a}
BS5	42 ± 13^{b}	26 ± 3^{cd}	51 ± 4^{c}	59 ± 8^{cd}	45 ± 6^{c}	48 ± 2^{d}	30 ± 5^{d}	28 ± 3^{e}
BS10	62 ± 3^{b}	31 ± 1^{b}	60 ± 1^{b}	80 ± 2^{b}	70 ± 3^{bc}	60 ± 1^{c}	41 ± 2^{bc}	34 ± 2^{cd}
BS15	60 ± 2^{b}	36± 1^{ab}	69 ± 1^{a}	84 ± 1^{b}	107 ± 2^{a}	69 ± 1^{b}	42 ± 2^{bc}	38 ± 1^{bc}
EAA*	16	30	61	48	23	41	25	40

General linear model with semolina pasta excluded from the calculation								
Type of protein powders								
Egg white	72^{a}	30^{a}	56^{a}	89^{a}	55^{a}	61^{a}	50^{a}	39^{a}
Soy protein	75^{a}	25^{b}	51^{b}	88^{a}	56^{a}	52^{b}	44^{b}	28^{b}
Level of protein powders								
0	137^{a}	7^{d}	23^{d}	127^{a}	2^{d}	32^{d}	64^{a}	14^{c}
5	50^{b}	28^{c}	54^{c}	68^{b}	58^{c}	53^{c}	37^{c}	34^{b}
10	57^{b}	35^{b}	66^{b}	79^{b}	73^{b}	68^{b}	44^{b}	42^{a}
15	50^{b}	39^{a}	71^{a}	79^{b}	90^{a}	73^{a}	43^{bc}	45^{a}

*Recommendation of daily amino acid intake for adult human by WHO et al. (2007). BE5, BE10, BE15: Pasta prepared from 100% banana flour with 5, 10, 15 g addition of egg white protein powder/100 g flour. BS5, BS10, BS15: Pasta prepared from 100% banana flour with 5, 10, 15 g addition of soy protein isolate/100 g flour. Results in the table represent the mean of triplicate measurements. Mean ± standard deviation. Values within a column within a group followed by the same superscript letter are not significantly different from each other ($p > 0.05$), according to Tukey's test.

Amino acid scores and protein digestibility-corrected amino acid scores can be seen in Table 8.4. Semolina pasta had several limiting EAA (Essential Amino Acid) compared to WHO reference, with valine as the most limiting EAA. Banana pasta had most limiting EAA and showed lack of some EAA content: valine (val), isoleucine (ile), and cysteine (cys) + methionine (met), corresponding with WHO recommendation. Egg white protein inclusion in banana pasta gave a better AAS value compared with soy protein isolate and, with a level of minimum 10% egg white protein fortification, fulfilled AA recommendation for daily adult intake showed by no limiting AAS (all values > 1).

Protein digestibility-corrected amino acid score of cooked pasta samples ranged between 0.07 to 1.00 (Table 8.4). Banana pasta sample had the lowest value because of its limiting AAS and low *in vitro* protein digestibility. Interestingly, all enriched banana pasta had higher PDCAAS value compared to semolina pasta while this pasta control had highest *in vitro* protein digestibility among all the pasta samples. This result showed that enriched banana pasta performed as a better protein quality source compared to semolina pasta. Egg white protein also showed as a better protein quality compared to soy protein isolate, forming a higher PDCAAS value and, at level 15% egg white enrichment, exhibited the maximum value of PDCAAS. Egg white protein has been used as a protein quality standard and its PDCAAS value has similar optimal value as milk protein (Corgneau et al., 2019; Layman and Rodriguez, 2009).

Table 8.4 Amino acid scores (AAS) (mg per g protein) and protein digestibility corrected amino acid scores (PDCAAS) of semolina pasta and banana pasta

Type of pasta	Amino Acid								
	His	Ile	Leu	Lys	Cys + Met	Phe + Tyr	Thr	Val	PDCAAS
Semolina	3.57 ± 0.17^{b}	0.80 ± 0.04^{cd}	0.87 ± 0.04^{bc}	0.96 ± 0.05^{d}	5.62 ± 0.32^{a}	1.44 ± 0.08^{c}	1.31 ± 0.06^{cd}	**0.73 ± 0.03**^{de}	0.63 ± 0.03^{c}
Banana	8.57 ± 0.46^{a}	0.22 ± 0.02^{d}	0.38 ± 0.02^{d}	2.65 ± 0.15^{a}	**0.10 ± 0.01**^{d}	0.78 ± 0.03^{e}	2.57 ± 0.12^{a}	0.35 ± 0.02^{f}	0.07 ± 0.01^{e}
BE5	3.72 ± 0.15^{b}	1.03 ± 0.05^{bc}	0.94 ± 0.07^{bc}	1.61 ± 0.25^{b}	3.09 ± 0.18^{b}	1.41 ± 0.10^{c}	1.75 ± 0.26^{b}	**1.01 ± 0.07**^{b}	0.69 ± 0.05^{bc}
BE10	3.32 ± 0.70^{b}	1.32 ± 0.02^{a}	**1.16 ± 0.01**^{a}	1.63 ± 0.17^{b}	3.29 ± 0.32^{b}	1.82 ± 0.02^{ab}	1.87 ± 0.15^{b}	1.27 ± 0.02^{a}	0.87 ± 0.00^{a}
BE15	2.49 ± 0.20^{b}	1.39 ± 0.03^{a}	**1.21 ± 0.04**^{a}	1.52 ± 0.05^{bc}	3.15 ± 0.17^{b}	1.90 ± 0.07^{a}	1.77 ± 0.08^{b}	1.30 ± 0.03^{a}	0.94 ± 0.03^{a}
BS5	2.64 ± 0.80^{b}	**0.85 ± 0.11**^{c}	0.84 ± 0.07^{c}	1.22 ± 0.17^{cd}	1.94 ± 0.27^{c}	1.17 ± 0.05^{d}	1.20 ± 0.20^{d}	0.70 ± 0.08^{e}	0.51 ± 0.06^{d}
BS10	3.86 ± 0.19^{b}	1.04 ± 0.05^{b}	**0.99 ± 0.02**^{b}	1.67 ± 0.06^{b}	3.05 ± 0.12^{bc}	1.47 ± 0.03^{c}	1.65 ± 0.06^{bc}	0.84 ± 0.04^{cd}	0.65 ± 0.03^{bc}
BS15	3.72 ± 1.19^{b}	1.20 ± 0.04^{ab}	**1.13 ± 0.01**^{a}	1.76 ± 0.03^{b}	4.65 ± 0.94^{a}	1.68 ± 0.01^{b}	1.66 ± 0.06^{bc}	0.95 ± 0.03^{bc}	0.74 ± 0.02^{b}
General linear model with semolina pasta excluded from the calculation									
Type of protein powders									
Egg white	4.52^{a}	0.99^{a}	0.93^{a}	1.85^{a}	2.41^{a}	1.48^{a}	1.99^{a}	0.98^{a}	0.64^{a}
Soy protein	4.70^{a}	0.83^{b}	0.84^{b}	1.82^{a}	2.43^{a}	1.27^{b}	1.78^{b}	0.71^{b}	0.49^{b}
Level of protein powders									
0	8.57^{a}	0.22^{d}	0.38^{d}	2.65^{a}	0.10^{d}	0.78^{d}	2.57^{a}	0.35^{c}	0.07^{d}
5	3.18^{b}	0.94^{c}	0.89^{c}	1.42^{b}	2.52^{c}	1.29^{c}	1.47^{c}	0.85^{b}	0.60^{c}
10	3.59^{b}	1.18^{b}	1.08^{b}	1.65^{b}	3.17^{b}	1.65^{b}	1.76^{b}	1.06^{a}	0.76^{b}
15	3.10^{b}	1.29^{a}	1.17^{a}	1.64^{b}	3.90^{a}	1.79^{a}	1.72^{bc}	1.13^{a}	0.84^{a}

Numbers in bold show limiting value of AAS; BE5, BE10, BE15: Pasta prepared from 100% banana flour with 5, 10, 15 g addition of egg white protein powder/100 g flour. BS5, BS10, BS15: Pasta prepared from 100% banana flour with 5, 10, 15 g addition of soy protein isolate/100 g flour. Results in the table represent the mean of triplicate measurements. Mean ± standard deviation. Values within a column within a group followed by the same superscript letter are not significantly different from each other ($p > 0.05$), according to Tukey's test.

8.4 Conclusion

The addition of egg white protein and soy protein isolate into banana pasta altered the total phenolic content and antioxidant capacities compared to semolina pasta and pure banana pasta. Soy protein isolate increased TPC, ferric reducing/antioxidant power and ABTS radical scavenging capacity of banana pasta by the increasing level of fortification. On the contrary, egg white protein lowered phenolic content and antioxidant capacity values with increasing egg white protein addition.

Glycaemic properties of banana pasta were also affected by the type of protein addition. Egg white protein inhibited reducing sugars released in fortified banana pasta, while soy protein isolate did not have significant impact on starch digestion properties. Protein enrichment in banana pasta also altered protein digestibility. Protein digestibility increased along with the increased protein inclusion in banana pasta. Amino acid profiles of enriched banana pasta also enhanced and met daily requirement for adult human with optimum protein digestibility-corrected amino acid score. These results show that soy protein isolate and egg white protein addition into gluten-free pasta formulation based on banana flour improved the nutritional and digestibility properties of banana pasta better than those of semolina pasta. The optimum protein addition to enhancing the nutritional quality of gluten-free banana pasta was 5% because it gave the optimum TPC and antioxidant activities, similar starch digestibility and similar protein quality among other formulations.

Chapter 9

Effect of Egg White Protein and Soy Protein Fortification on Nutritional Quality and Sensory Properties of Gluten-Free Pasta Made of Cassava-Banana Composite Flour

Abstract

This study aimed to evaluate the effect of egg white protein and soy protein isolate addition on the nutritional quality, digestibility properties, protein digestibility corrected amino acid (PDCAAS), and sensory acceptance of gluten-free pasta based on cassava and banana flours. Banana flour was blended with cassava flour at a ratio of 75:25 (w/w), then egg white protein or soy protein isolate were added at the following rates 0, 5, 10, and 15 g/100 g flour. The gluten-free pasta formulation was extruded using a pasta making machine fitted with spaghetti die and 70% water addition (w/v). Cooked pasta samples were analysed for total phenolic content (TPC), antioxidant activity, amino acid profiles, protein content, starch digestibility, protein digestibility and PDCAAS was calculated. Addition of both proteins decreased starch digestibility, increased protein digestibility, improved amino acid profile, and PDCASS whereas only soy protein isolate enhanced the TPC and antioxidant capacity of the gluten-free pasta. Gluten-free banana-cassava pasta with added egg white powder had better customer acceptance and purchase intent compared to soy protein isolate inclusion.

Keywords: banana flour, cassava flour, gluten-free pasta, nutritional quality, sensory evaluation

9.1 Introduction

The inclusion of protein into gluten-free products has been studied to try to improve the physical properties and nutritional quality. Egg white protein is the most common protein source used for enrichment, alongside with soy protein isolate as a plant-origin protein source (Zhang et al., 2020). Soy protein isolate has been reported to have functional properties (emulsifier and stabiliser) and bioactivities such as reduce blood pressure, reduce diabetic, and high antioxidant activities (Chen, Sun-Waterhouse, Zhang, Zhao, and Sun, 2020).

Soy protein and egg white protein inclusion in gluten-free pasta production have been reported to improve the quality of gluten-free pasta (Detchewa et al., 2016). The effect of soy protein isolate on rice gluten-free pasta increased tensile strength and firmness which led to better pasta quality (Detchewa et al., 2016). The effect of egg white protein in gluten-free rice pasta also enhanced cooking properties and textural properties (chewiness and firmness) (Witek et al., 2020). However, there are only a few studies that have evaluated the effect of the addition of these protein sources on the nutritional quality and digestibility properties of gluten-free pasta.

Nutritional quality assessment includes evaluation of the protein quality of food products and its ability to fulfil an individuals amino acid requirement. The amino acid requirement is determined by analysing the amino acid score, protein efficiency ratio, *in vitro* protein digestibility, net protein utilization, net protein retention, *in vivo* protein digestibility, biological value, and PDCAAS (Desai et al., 2018a). Several researchers have discussed protein digestibility in gluten-free pasta based on millet (Cordelino et al., 2019), faba-oat (Duta et al., 2019), sorghum, maize, rice, and cassava (Palavecino, Ribotta, León, and Bustos, 2019), and brown rice (Rafiq et al., 2017). Duta et al. (2019) studied the protein digestibility of various commercial gluten-free pastas that also used egg white protein or soy flour in the formulation. However, there is a scarcity of studies that evaluate the PDCAAS of cereal products, especially in gluten-free pasta product.

The protein digestibility-corrected amino acid score (PDCAAS) has been used to measure the quality of protein in food products as suggested by the FAO/WHO Expert Consultation since 1991 (Han et al., 2018a). This method corrects amino acid scores by the protein composition and protein digestibility of the tested food products (Shaheen, Islam, Munmun, Mohiduzzaman, and Longvah, 2016). An advanced measurement for protein quality has been recommended using the digestible indispensable amino acid score (DIAAS) that uses true ileal amino acid digestibility for a specific food product. However, the information on the ileal amino acid digestibility is still limited and needs to be determined by an *in vivo* experiment (Han et al., 2018a).

Banana flour utilisation in gluten-free pasta had been evaluated regarding the antioxidant compounds on starch digestibility and showed decreasing in the glycaemic index (Agama-Acevedo et al., 2019). Banana flour, alongside cassava flour and rice flour, are the most common materials used in many studies regarding gluten-free pasta. Banana flour has been reported to have bioactive compounds (phytosterol, total phenolic, and antioxidant activities) that may have anti-cancer activity, reducing human blood level and cholesterol (Cheok et al., 2018). Cassava flour utilisation in gluten-free pasta is limited by its low protein content and starch characteristics (Odey and Lee, 2020). A previous study showed a portion of cassava flour in gluten-free pasta formulation that gave optimum quality is 25 g/100 g (Chapter 5).

Beyond the nutritional values of food products, a sensory evaluation can give a comprehensive evaluation of how an effort to provide healthy products can affect consumer acceptance. Detchewa et al. (2016) found that a 5% soy protein isolate addition to rice flour pasta had the best sensory acceptability (6.16 out of 9) compared to other rice pasta formulations (0-10 % soy protein isolate addition), but this was still lower than the commercial wheat pasta acceptance score (7.46 out of 9). Zandonadi et al. (2012) revealed that gluten-free pasta made with unripe plantain and 31% egg white had no difference in sensory acceptance compared to a wheat pasta as a control (6.13 and 5.93 out of 9, respectively).

This study aimed to evaluate the nutritional quality (total phenolic, antioxidant activities, and amino acid profiles) and digestibility properties (starch and protein digestibility), alongside the PDCAAS and sensory evaluation of gluten-free pasta based on cassava and banana flours enriched with soy protein isolate or egg white protein.

9.2 Materials and methods

9.2.1 Materials

Described in section 3.1.

9.2.2 Pasta preparation

Described in section 3.2.3. and 3.2.5

9.2.3 Total phenolic content and antioxidant capacity

Described in section 3.2.15.

9.2.4 *In vitro* starch digestibility

Described in section 3.2.20

9.2.5 Protein content for *in vitro* protein digestibility determination

Described in section 3.2.10.

9.2.6 *In vitro* protein digestibility

Described in section 3.2.21.

9.2.7 Amino acid profiles, amino acid score (AAS) and protein digestibility-corrected amino acid score (PDCAAS)

Described in section 3.2.16 and 3.2.22.

9.2.8 Sensory evaluation

Described in section 3.2.23.

9.2.9 Statistical analysis

Described in section 3.2.24.

9.3 Results and discussion

9.3.1 Total phenolic content (TPC) and antioxidant capacities

A comparison of TPC and antioxidant activities (FRAP and ABTS) of gluten-free pasta based on banana-cassava flour and semolina pasta showed significant differences as can be seen in table 9.1. The banana-cassava pasta had a higher TPC and antioxidant capacities compared to semolina pasta. A high TPC and antioxidant capacities in banana flour produced high TPC and antioxidant capacities of gluten-free pasta even it was blended with cassava flour that had low quality of TPC and antioxidant capacities. This showed that incorporation of 25% cassava flour into the gluten-free pasta formulation did not give a significant deterioration of TPC or antioxidant ability in gluten-free pasta based on banana flour. Similar results were found in the previous study regarding banana pasta evaluation on nutritional qualities including TPC and antioxidant activities (Chapter 8).

The type of protein addition to gluten-free pasta gave a significant difference in TPC and antioxidant capacities. Soy protein isolate increased the TPC and antioxidant properties of gluten-free pasta, egg white protein decreased the TPC and antioxidant value with increasing level inclusion, this is because soy protein isolate has a higher TPC and antioxidant activities than egg white protein. Egg white protein also had a lower TPC and antioxidant capacities compared to banana flour. Soy protein isolate has high antioxidant ability mainly due to its amino acid profiles, the length of the peptide chains, sequence, and interaction of the peptide-liposomal membrane (Chen et al., 2020; Lorenzo et al., 2018).

Table 9.1 TPC, FRAP and ABTS values of gluten-free banana-cassava pasta compared to semolina pasta

Sample	TPC (mg GAE/ 100g DM)	FRAP (mmol Fe^{2+}/100g DM)	ABTS (µmol TE/100g DM)
Raw materials			
Semolina flour	73.80 ± 0.78	0.15 ± 0.02	0.67 ± 0.02
Banana flour	116.45 ± 4.75	1.14 ± 0.00	1.46 ± 0.02
Cassava flour	46.69 ± 1.20	0.87 ± 0.02	0.54 ± 0.01
Soy protein isolate	261.26 ± 2.50	1.06 ± 0.00	2.72 ± 0.01
Egg white protein	70.19 ± 1.85	0.49 ± 0.00	0.11 ± 0.01
Pasta samples			
Semolina	55.73 ± 0.73^{e}	0.10 ± 0.00^{g}	0.31 ± 0.02^{g}
BC	66.31 ± 1.79^{d}	1.05 ± 0.03^{a}	0.91 ± 0.02^{c}
BCE5	55.14 ± 1.77^{e}	0.85 ± 0.02^{de}	0.75 ± 0.02^{d}
BCS5	71.50 ± 0.69^{c}	0.92 ± 0.02^{cd}	0.91 ± 0.01^{c}
BCE10	52.32 ± 1.41^{e}	0.79 ± 0.04^{e}	0.69 ± 0.02^{e}
BCS10	88.13 ± 2.46^{b}	0.96 ± 0.02^{bc}	1.33 ± 0.01^{b}
BCE15	41.57 ± 2.53^{f}	0.70 ± 0.03^{f}	0.58 ± 0.01^{f}
BCS15	100.91 ± 1.53^{a}	1.00 ± 0.02^{ab}	1.50 ± 0.01^{a}
General linear model with semolina pasta excluded from the calculation			
Type of protein powders			
Egg white protein	53.83^{b}	0.85^{b}	0.73^{b}
Soy protein isolate	81.71^{a}	0.98^{a}	1.16^{a}
Level of protein powders			
0%	66.31^{b}	1.04^{a}	0.91^{c}
5%	63.32^{b}	0.89^{b}	0.83^{d}
10%	70.23^{a}	0.87^{b}	1.01^{b}
15%	71.24^{a}	0.85^{b}	1.04^{a}

BC: Pasta prepared from 75% banana flour and 25% cassava flour. BCE5, BCE10, BCE15: BC with 5, 10, 15 g addition of egg white protein powder/100 g flour. BCS5, BCS10, BCS15: BE with 5, 10, 15 g addition of soy protein isolate/100 g flour. Results in the table represent the mean of triplicate measurements. Mean ± standard deviation. Values within a column within a group followed by the same superscript letter are not significantly different from each other (p > 0.05), according to Tukey's test.

9.3.2 *In vitro* starch digestibility

Gluten-free pasta made from banana and cassava flour without any protein enrichment had no difference in total area under the curve (AUC) compared to semolina pasta, showing a similar level of *in vitro* starch digestibility (Figure 9.1). Both egg white protein and soy protein inclusion reduced the starch digestibility of the gluten-free pasta significantly. A lower starch digestibility due to protein addition (oat protein isolate, faba protein isolate, and chickpea protein isolate) to gluten-free pasta also was observed by other studies based on oat starch (Duta et al., 2019), and rice flour (Sofi, Singh, Chhikara, and Panghal, 2020).

An increasing level of egg white protein addition led to lower starch digestibility in gluten-free pasta, while the level of soy protein isolate addition (5, 10, or 15%) did not give any differences. These results showed that egg white protein gives a greater effect in reducing the starch digestibility of gluten-free pasta compared to soy protein isolate. This pattern is similar to the previous study regarding the influence of soy protein isolate and egg white protein on the starch digestibility of banana pasta (Chapter 8). Similar results were found regarding the effect of different types of protein (oat protein concentrate and faba protein concentrate) and level of protein addition (18% and 35%) on the starch digestibility of gluten-free pasta based on oat starch. Oat protein isolate inclusion reduced starch digestibility compared to the gluten-free oat pasta control, but the different addition levels (18% and 35%) did not have a different hydrolysis index or glycaemic index (Duta et al., 2019). This indicates that various types of protein might have a specific/optimum range of addition to reduce the starch digestibility of pasta samples.

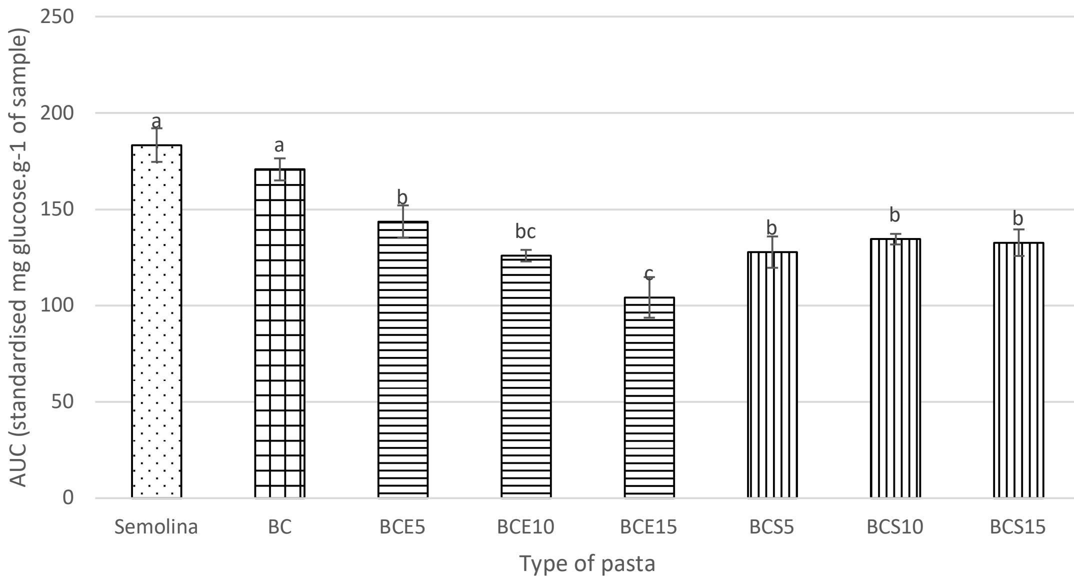


Figure 9.1 Area under curve (iAUC) values of banana pasta and banana pasta with protein addition
(BC = Pasta made from 75% banana flour and 25% cassava flour, BCE5, BEC10, BCE15 = BC with 5, 10, 15 g egg white protein/100 g flour, BS5, BS10, BS15 = BC with 15 g soy protein/100 g flour) compared to semolina pasta as a control. Different letters above the bars showed significantly different from each other (*p* > 0.05), according to Tukey's test.

9.3.3 *In vitro* protein digestibility

Protein content, protein digestibility and protein availability of pasta samples varied, as shown in table 9.2. Gluten-free pasta based on banana and cassava flours had a lower protein content (1.79%), lower protein digestibility (70.43%), and lower protein availability (2.24%) compared to semolina pasta (12.26%, 85.81 and 10.52%, respectively). The protein incorporation increased the protein content significantly, followed by the increase of protein digestibility and protein availability. Both types of protein raised the protein digestibility as there was proportionately more protein in the gluten-free pasta. Other studies have also reported protein digestibility enhancement in gluten-free pasta with protein incorporation (Duta et al., 2019; Manoj Kumar et al., 2019; Sofi et al., 2020) and in wheat-based pasta with plant protein isolate (soy, pea, and corn protein isolate) addition (Khatkar and Kaur, 2018; Osipova, Koryachkina, Koryachkin, Seregina, and Zhugina, 2019).

Table 9.2 Protein content, protein digestibility and protein availability of gluten-free pasta and semolina pasta

Type of pasta	Protein (% db)	Protein Digestibility (%)	Protein Availability (%)
Semolina	12.26 ± 0.09^{c}	85.81 ± 0.10^{a}	10.52 ± 0.09^{c}
BC	1.79 ± 0.05^{g}	70.43 ± 0.46^{e}	2.24 ± 0.05^{f}
BCE5	6.81 ± 0.04^{f}	73.38 ± 0.55^{d}	5.00 ± 0.07^{e}
BCS5	6.78 ± 0.04^{f}	75.01 ± 0.28^{cd}	5.08 ± 0.05^{e}
BCE10	10.25 ± 0.16^{e}	75.92 ± 1.28^{c}	7.78 ± 0.23^{d}
BCS10	10.87 ± 0.07^{d}	76.34 ± 0.36^{c}	8.30 ± 0.06^{c}
BCE15	13.56 ± 0.07^{b}	76.64 ± 0.69^{bc}	10.39 ± 0.15^{b}
BCS15	14.22 ± 0.09^{a}	78.09 ± 0.10^{b}	11.10 ± 0.09^{a}
General linear model with semolina pasta excluded from the calculation			
Type of protein powders			
Egg white protein	8.10^{b}	74.09^{b}	6.35^{b}
Soy protein isolate	8.41^{a}	74.97^{a}	6.68^{a}
Level of protein powders			
0%	1.79^{d}	70.43^{d}	2.24^{d}
5%	6.79^{c}	74.20^{c}	5.04^{c}
10%	10.56^{b}	76.13^{b}	8.04^{b}
15%	13.89^{a}	77.36^{a}	10.74^{a}

BC: Pasta prepared from 75% banana flour and 25% cassava flour. BCE5, BCE10, BCE15: BC with 5, 10, 15 g addition of egg white protein powder/100 g flour. BCS5, BCS10, BCS15: BE with 5, 10, 15 g addition of soy protein isolate/100 g flour. Results in the table represent the mean of triplicate measurements. Mean ± standard deviation. Values within a column within a group followed by the same superscript letter are not significantly different from each other ($p > 0.05$), according to Tukey's test.

Soy protein gave a higher protein digestibility compared to egg white protein, this is because the soy protein isolate forms a weaker protein network in the pasta. Laleg, Barron, Santé-Lhoutellier, Walrand, and Micard (2016a) found that wheat pasta enriched with faba protein had a higher protein digestibility (46%) compared to egg protein (39%) which was also related to a weaker protein network in the faba enriched pasta (18% covalent link) than in the egg protein powder enriched pasta (32% covalent link). These findings were also observed in the previous research on protein enrichment in gluten-free pasta based on banana flour (Chapter 8), which illustrated that 25% cassava flour incorporation in gluten-free pasta production did not alter the protein digestibility properties.

9.3.4 Protein digestibility-corrected amino acid score (PDCAAS)

Semolina pasta had four EAAs that did not meet the EAA daily consumption recommendation (Table 9.3) namely isoleucine (ile), leucine (leu), lysine (lys), and valine (val). Banana-cassava gluten-free pasta EAA composition (Table 9.3), only had 3 out of 8 groups of EAA that met the daily recommendation (histidine (his), threonine (thr), and lysine). However a 5% protein inclusion (egg white protein or soy protein isolate) to gluten-free pasta based on cassava and banana flours was enough to improve the amino acid profiles to meet the EAA recommendation for the daily diet (FAO, 2013). Egg white protein gave a better valine enrichment, while soy protein isolate led to a higher level of histidine.

Chen et al. (2020) reported that amino acid composition affected the antioxidant activities, especially tyrosine (tyr), cysteine (cys), methionine (met), histidine, lysine, and phenylalanine (phe) content. Banana-cassava pasta had a rich histidine and lysine composition that may contribute to its high antioxidant properties. The soy protein isolate-enriched pasta had a high content of amino acids that have known antioxidant activities (cysteine, methionine, histidine, and lysine)(Chen et al., 2020). This led to the soy protein isolate enriched pasta having higher antioxidant capacities compared to the egg white protein-enriched gluten-free pasta.

Table 9.3 Comparison of amino acid profiles in semolina pasta and banana-cassava pasta with daily adult intake recommendation (mg of amino acid per g protein)

Type of pasta	Amino Acid							
	His	Ile	Leu	Lys	Cys + Met	Phe + Tyr	Thr	Val
Semolina	57 ± 3^{c}	24 ± 1^{d}	53 ± 3^{d}	46 ± 2^{e}	129 ± 7^{a}	59 ± 3^{e}	33 ± 2^{d}	29 ± 1^{c}
BC	160 ± 6^{a}	6 ± 1^{e}	23 ± 2^{e}	148 ± 5^{a}	1 ± 1^{e}	33 ± 1^{f}	75 ± 3^{a}	14 ± 1^{d}
BCE5	36 ± 6^{d}	39 ± 4^{bc}	72 ± 5^{bc}	61 ± 7^{d}	59 ± 12^{d}	71 ± 6^{bcd}	37 ± 6^{cd}	48 ± 4^{a}
BCE10	45 ± 1^{cd}	40 ± 2^{b}	71 ± 2^{bc}	78 ± 2^{c}	75 ± 1^{bcd}	73 ± 1^{bc}	43 ± 1^{c}	51 ± 2^{a}
BCE15	40 ± 12^{d}	41 ± 1^{ab}	75 ± 4^{b}	73 ± 6^{cd}	79 ± 5^{bc}	78 ± 2^{ab}	43 ± 2^{c}	52 ± 1^{a}
BCS5	50 ± 3^{cd}	38 ± 3^{bc}	72 ± 5^{bc}	75 ± 5^{c}	73 ± 13^{bcd}	68 ± 5^{cd}	38 ± 2^{cd}	40 ± 3^{b}
BCS10	75 ± 3^{b}	46 ± 3^{a}	86 ± 4^{a}	104 ± 3^{b}	92 ± 3^{b}	86 ± 3^{a}	52 ± 1^{b}	48 ± 3^{a}
BCS15	59 ± 1^{c}	33 ± 1^{c}	64 ± 1^{c}	78 ± 2^{c}	71 ± 2^{cd}	64 ± 0de	39 ± 1^{cd}	35 ± 1^{bc}
EAA*	16	30	61	48	23	41	25	40

General linear model with semolina pasta excluded from the calculation								
Type of protein powders								
Egg white	70^{b}	31^{a}	60^{a}	90^{b}	54^{a}	64^{a}	49^{a}	41^{a}
Soy protein	89^{a}	31^{a}	61^{a}	101^{a}	59^{a}	63^{a}	51^{a}	34^{b}
Level of protein powders								
0	160^{a}	6^{b}	23^{b}	148^{a}	1^{c}	33^{c}	75^{a}	14^{b}
5	43^{c}	38^{a}	72^{a}	68^{c}	66^{b}	70^{b}	37^{c}	44^{a}
10	60^{b}	43^{a}	79^{a}	91^{b}	83^{a}	80^{a}	47^{b}	49^{a}
15	49^{bc}	37^{a}	70^{a}	75^{c}	75^{ab}	71^{ab}	41^{c}	43^{a}

*Recommendation of daily amino acid intake for adult human by WHO et al. (2007). BC: Pasta prepared from 75% banana flour and 25% cassava flour. BCE5, BCE10, BCE15: BC with 5, 10, 15 g addition of egg white protein powder/100 g flour. BCS5, BCS10, BCS15: BE with 5, 10, 15 g addition of soy protein isolate/100 g flour. Results in the table represent the mean of triplicate measurements. Mean ± standard deviation. Values within a column within a group followed by the same superscript letter are not significantly different from each other ($p > 0.05$), according to Tukey's test.

Table 9.4 Amino acid scores (AAS) (mg per g protein) and protein digestibility corrected amino acid scores (PDCAAS) of semolina pasta and banana-cassava pasta

Type of pasta	Amino Acid								
	His	Ile	Leu	Lys	Cys + Met	Phe + Tyr	Thr	Val	PDCAAS
Semolina	3.57 ± 0.17^{c}	0.80 ± 0.04^{d}	0.87 ± 0.05^{d}	0.96 ± 0.05^{e}	4.10 ± 1.24^{a}	2.51 ± 0.75^{a}	1.31 ± 0.06^{d}	**0.73 ± 0.03^{c}**	0.63 ± 0.03^{d}
BC	10.01^{a} ± 0.34	0.19 ± 0.04^{e}	0.37 ± 0.04^{e}	3.09 ± 0.04^{a}	**0.06 ± 0.03^{c}**	0.80 ± 0.02^{c}	3.00 ± 0.12^{a}	0.35 ± 0.03^{d}	0.04 ± 0.02^{e}
BCE5	2.26 ± 0.40^{d}	1.29 ± 0.12^{bc}	**1.19 ± 0.08^{bc}**	1.27 ± 0.08^{d}	2.56 ± 0.52^{b}	1.73 ± 0.13^{ab}	1.47 ± 0.23^{cd}	1.20 ± 0.03^{a}	0.87 ± 0.10^{ab}
BCE10	2.79 ± 0.05^{cd}	1.32 ± 0.05^{b}	**1.16 ± 0.03^{bc}**	1.62 ± 0.03^{c}	3.26 ± 0.03^{ab}	1.79 ± 0.04^{ab}	1.73 ± 0.02^{c}	1.26 ± 0.03^{a}	0.88 ± 0.05^{a}
BCE15	2.51 ± 0.73^{d}	1.38 ± 0.04^{ab}	**1.23 ± 0.06^{b}**	1.51 ± 0.06^{cd}	3.43 ± 0.21^{ab}	1.90 ± 0.04^{ab}	1.72 ± 0.07^{c}	1.31 ± 0.03^{a}	0.94 ± 0.02^{a}
BCS5	3.15 ± 0.19^{cd}	1.26 ± 0.08^{bc}	1.18 ± 0.09^{bc}	1.55 ± 0.09^{c}	3.17 ± 0.55^{ab}	1.67 ± 0.13^{b}	1.52 ± 0.10^{cd}	**1.00 ± 0.03^{b}**	0.75 ± 0.07^{bc}
BCS10	4.66 ± 0.17^{b}	1.53 ± 0.10^{a}	1.41 ± 0.07^{a}	2.17 ± 0.07^{b}	4.00 ± 0.12^{ab}	2.09 ± 0.08^{ab}	2.06 ± 0.05^{b}	**1.19 ± 0.03^{a}**	0.91 ± 0.08^{a}
BCS15	3.66 ± 0.07^{c}	1.11 ± 0.03^{c}	1.05 ± 0.01	1.63 ± 0.01^{c}	3.08 ± 0.07^{ab}	1.56 ± 0.01^{bc}	1.57 ± 0.05^{cd}	**0.87 ± 0.03^{bc}**	0.68 ± 0.02^{cd}
General linear model with semolina pasta excluded from the calculation									
Type of protein powders									
Egg white	4.39^{b}	1.04^{a}	0.99^{a}	1.87^{b}	2.33^{a}	1.56^{a}	1.98^{a}	1.03^{a}	0.70^{a}
Soy protein	5.37^{a}	1.02^{a}	1.00^{a}	2.11^{a}	2.58^{a}	1.53^{a}	2.03^{a}	0.85^{b}	0.62^{b}
Level of protein powders									
0	10.01^{a}	0.019^{b}	0.37^{b}	3.09^{a}	0.07^{c}	0.80^{c}	3.00^{a}	0.35^{b}	0.13^{b}
5	2.70^{c}	1.27^{a}	1.18^{a}	1.41^{c}	2.87^{b}	1.70^{b}	1.49^{c}	1.10^{a}	0.81^{a}
10	3.72^{b}	1.42^{a}	1.29^{a}	1.90^{b}	3.63^{a}	1.94^{a}	1.89^{b}	1.23^{a}	0.90^{a}
15	3.08^{bc}	1.25^{a}	1.14^{a}	1.57^{c}	3.25^{ab}	1.73^{ab}	1.65^{c}	1.09^{a}	0.81^{a}

BC: Pasta prepared from 75% banana flour and 25% cassava flour. BCE5, BCE10, BCE15: BC with 5, 10, 15 g addition of egg white protein powder/100 g flour. BCS5, BCS10, BCS15: BE with 5, 10, 15 g addition of soy protein isolate/100 g flour. Results in the table represent the mean of triplicate measurements. Mean ± standard deviation. Values within a column within a group followed by the same superscript letter are not significantly different from each other ($p > 0.05$), according to Tukey's test.

The PDCAAS of gluten-free pasta enriched with egg white protein or soy protein isolate were higher than semolina pasta or gluten-free pasta without any protein addition (Table 9.4). Banana pasta had PDCAAS of 0.63 with valine as a limited amino acid score (AAS). Banana-cassava gluten-free pasta had the lowest PDCAAS (0.03) that was also limited by valine (AAS 0.03). The protein inclusion enhanced PDCAAS ranged from 0.68 to 0.94 and was higher than the semolina pasta score. Egg white protein-enriched banana-cassava pasta had a higher PDCAAS (0.70) compared to soy protein isolate-enriched banana-cassava pasta (0.62). The level of protein addition (5-15%) did not have a significant effect on PDCAAS. These results showed that the 5% protein addition was the optimum level to gain a good quality of protein in gluten-free pasta based on banana and cassava flours.

9.3.5 Sensory properties

The sensory evaluation result of banana-cassava pasta showed significant acceptance compared to semolina pasta (Table 9.5). All the gluten-free pasta tested had lower overall scores (4.73 to 5.65 out of 9) compared to semolina pasta (7.19 out of 9). The texture of gluten-free pasta (5% egg white protein addition) was the only attribute that had a similar score with semolina pasta.

Egg white protein-enriched banana-cassava pasta had a better overall perception than the soy protein isolate-enriched pasta(5.65 and 4.73 out of 9, respectively). Other research into the sensory qualities of banana pasta enriched with 31.5% egg white showed a similar overall score of 6.13 out of 9 (Zandonadi et al., 2012). Detchewa et al. (2016) reported a better overall sensory acceptance of soy protein isolate addition (0-10%) in gluten-free pasta based on rice flour (overall acceptance of 6.10 to 6.60 out of 9, but this was still lower than the semolina pasta control (8.00 out of 9). A 15% protein addition did not give a better overall acceptance than a 5% protein addition in gluten-free pasta. These results illustrate that a banana-cassava gluten-free pasta with egg white protein addition had moderate sensory acceptance (neither like nor dislike to slightly like). Soy protein incorporation in gluten-free pasta had low overall perception (slightly dislike to neither like nor dislike).

Table 9.5 Sensory properties of semolina pasta and banana-cassava pasta

Pasta	Appearance	Aroma	Texture	Flavour	Bitterness	Aftertaste	overall
Semolina	7.21± 1.34^{a}	6.49 ± 1.45^{a}	6.84 ± 1.19^{a}	6.62 ± 1.48^{a}	6.38 ± 1.30^{a}	6.76 ± 1.34^{a}	7.19 ± 1.13^{a}
BCE5	5.60 ± 1.62^{b}	5.03 ± 1.40^{b}	5.95 ± 1.53^{ab}	5.41 ± 1.67^{b}	5.35 ± 1.53^{b}	5.22 ± 1.65^{b}	5.54 ± 1.48^{b}
BCE15	5.49 ± 1.76^{b}	5.30 ± 1.56^{b}	5.81 ± 1.49^{b}	5.51 ± 1.74^{b}	5.27 ± 1.37^{b}	5.32 ± 1.73^{b}	5.65 ± 1.51^{b}
BCS5	3.97 ±1.66^{c}	4.65 ± 1.60^{b}	4.43 ± 1.82^{c}	5.05 ± 1.55^{b}	5.03 ± 1.52^{b}	4.92 ± 1.77^{b}	4.73 ± 1.50^{b}
BCS15	4.14 ± 1.21^{c}	5.05 ± 1.29^{b}	4.81 ± 1.68^{c}	4.87 ± 1.67^{b}	4.92 ± 1.36^{b}	4.92 ± 1.74^{b}	4.73 ± 1.50^{b}
General linear model ANOVA (semolina pasta excluded)							
Type of protein powders							
EWP	5.54054^{a}	5.16216^{a}	5.87838^{a}	5.45946^{a}	5.31081^{a}	5.27027^{a}	5.56757^{a}
SPI	4.05405^{b}	4.85135^{a}	4.62162^{b}	4.95946^{a}	4.97297^{a}	4.91892^{a}	4.72973^{b}
Level of protein powders							
5%	4.78378^{a}	4.83784^{a}	5.18919^{a}	5.22973^{a}	5.18919^{a}	5.06757^{a}	5.10811^{a}
15%	4.81081^{a}	5.17568^{a}	5.31081^{a}	5.18919^{a}	5.09459^{a}	5.12162^{a}	5.18919^{a}

Semolina: Pasta prepared from 100% semolina flour. BCE5, BCE15: Pasta prepared from 75% banana : 25% cassava flours with 5 or 15 g addition of EWP/100 g flour. BCS5, BCS15: Pasta prepared from 75% banana : 25% cassava flours with 5 or15 g addition of SPI/100 g flour. EWP : Egg white protein, SPI : Soy protein isolate. Results represent the mean of 37 panellists. Mean ± standard deviation. Values within a column followed by the same superscript letter are not significantly different from each other ($p > 0.05$), according to Tukey's test.

Panellists gave a higher score for appearance, texture, and overall liking of egg white protein-enriched pasta compared to soy protein isolate-enriched pasta. There were no significant differences for aroma, flavour, bitterness, and aftertaste of both protein enrichment in gluten-free pasta. The levels of protein addition (5% and 15%) did not show any different acceptance of all sensory test attributes.

Table 9.6 shows the overall texture and flavour perception varied for each of the pasta samples. Egg white protein, either 5% or 15% addition, showed a good perception (just about right) of texture (73% and 70%, respectively) and flavour (62% and 59%, respectively). Different texture and flavour perception were found for soy protein isolate addition. More than half panellists (54%) said that the texture was too soft texture with 15% soy protein isolate addition, and 46% panellists thought that texture of the same sample was just about right. The texture perception seems to be related to the textural properties of cooked pasta, especially for the extensibility results (Table 7.3, chapter 7). Gluten-free pasta with egg white protein addition had similar value (17.33 to 34.39 g) to semolina pasta (34.45 g), while soy protein isolate inclusion had lower extensibilities (9.54-23.06 g) that resulted in a too soft texture perception. For the flavour of soy protein addition, 46% panellists said that 5% soy protein addition was too weak, and 51% panellists scored a just about right flavour for banana-cassava pasta with 15% soy protein isolate addition.

Purchase intent (Table 9.6) of semolina pasta had a high score (81% of panellists were willing to buy). Gluten-free pasta based on banana and cassava flours with egg white protein inclusion had a moderate purchase intent (49% and 46%), while soy protein isolate had a low score of panellists wanted to buy the products (19% and 16%). These showed that gluten-free pasta, especially with egg white protein addition, had a potential to enter the market.

Table 9.6 Overall texture and flavour perception and purchase intent of pasta samples

Pasta	Texture (%)			Flavour (%)			Purchase intent (%)
	Too soft	JAR	Too hard	Too weak	JAR	Too strong	
Semolina	8	**81**	11	16%	**78**	5	81
BCE5	16	**73**	11	16	**62**	22	49
BCE15	8	**70**	22	22	**59**	19	46
BCS5	**54**	38	8	**46**	38	16	19
BCS15	43	**46**	11	22	**51**	27	16

JAR = just about right. BCE5, BCE15: Pasta prepared from 75% banana : 25% cassava flours with 5 or 15 g addition of egg white protein/100 g flour. BCS5, BCS15: Pasta prepared from 75% banana : 25% cassava flours with 5 or 15 g addition of soy protein isolate/100 g flour.

9.4 Conclusion

The addition of a protein source improved the nutritional quality (total phenolic content, antioxidant activities, and amino acid profiles) of the gluten-free banana-cassava pasta and when compared to semolina pasta. It also increased protein digestibility, reduced starch digestibility, and enhanced PDCAAS. Soy protein isolate was more effective than egg white protein at improving the TPC, antioxidant capacities, amino acid profiles, and increasing protein digestibility. Egg white protein supplementation gave a lower starch digestibility and had a better sensory acceptance compared to soy protein isolate inclusion. Future studies to improve the sensory acceptability of gluten-free pasta based on banana and cassava flours are needed to make it comparable to semolina pasta.

Chapter 10

General discussion and conclusion

10.1 Summary

This study illustrated that banana and cassava flours had promising physico-chemical and nutritional properties to be developed as alternative materials in gluten-free cereal products. Banana and cassava flours could be utilised in gluten-free pasta production with high fibre content and low starch digestibility properties. Soy protein isolate and egg white protein could be incorporated into gluten-free pasta based on banana and cassava flours to promote pasta quality and nutritional properties of alternative functional products. The addition of soy protein isolate and egg white protein increased protein content, improved cooking quality, and textural properties of gluten-free pasta based on banana and cassava flours. The results showed that protein sources inclusion significantly reduced starch digestibility, increased protein digestibility, total phenolic content (TPC), antioxidant capacities, and enhanced amino acid profiles. Sensory evaluation showed banana-cassava gluten-free pasta with egg white protein inclusion was acceptable. Soy protein isolate had higher TPC, antioxidant capacities, and protein digestibility, while egg white protein gave more impact on reduced starch digestibility.

10.2 Discussion

Banana and cassava flours had high dietary fibre content and low-fat content that may have health benefits to prevent obesity, diabetic and cardiovascular risk (Vitale et al., 2020). Chapter 4 revealed the physio-chemical and nutritional quality of banana and cassava flours and that these significantly differed from semolina flour. These two gluten-free flours had low protein and amylose content as role points to be concerned in gluten-free pasta production. The adequate protein content is needed to build a strong structure in gluten-free pasta, alongside with enhancing nutritional content (Gao et al., 2018; Woomer and Adedeji, 2020). High amylose content contributes to texture integrity and cooking quality, including low cooking loss of gluten-free pasta (Cordelino et al., 2019; Gao et al., 2018).

Chapter 5 presented physico-chemical properties, cooking quality, texture properties, and starch digestibility of gluten-free pasta made of different proportions of banana and cassava flours (0:100 to 100:0 with 25% interval). This experiment was intended to illustrate the gluten-free pasta characteristics based on banana and cassava flours without any functional additives and specific processing conditions. An initial trial using conventional semolina pasta production procedure (Foschia et al., 2014) did not produce any gluten-free pasta. Several trials using different water addition (50-70 ml/100 g flour) and different temperature (100°C) gained better results. All gluten-free pasta formulation had a lower cooking quality and texture properties compared to semolina pasta because of the lack of protein content. These results were similar to other studies using banana flour and cassava flour as pasta ingredients (Biernacka et al., 2020; Fiorda et al., 2013a; Oladunmoye et al., 2017; Sarawong et al., 2014a). The best formulations were gluten-free pasta made of 100% banana flour followed by 75% Banana flour : 25% Cassava flour formulation. These gluten-free pasta compositions had the best cooking properties, textural properties among other gluten-free pasta, and lower glycaemic properties compared to semolina pasta.

The effects of soy protein isolate and egg white protein addition on physicochemical and pasta quality of gluten-free pasta are presented in Chapter 6 and Chapter 7. Soy protein isolate and egg white protein incorporation in gluten-free pasta based on banana flour increased protein content, decreased moisture content, but did not affect dietary fibre composition (Chapter 6). The utilisation of 25% cassava flour alongside 75% banana flour in gluten-free pasta formulation showed similar trend in protein and moisture content proportion due to protein inclusion (Chapter 7). The adequate protein content in the gluten-free pasta mixture improved dough matrix build of starch-protein interaction that might entrap water molecules that resulted in reduced free water in gluten-free pasta (Desai et al., 2018b). Gluten-free pasta had significantly higher dietary fibre content then semolina pasta, and the protein incorporation did not alter the dietary fibre of gluten-free pasta. The resistant starch of gluten-free pasta reduced by the increase of protein inclusion due to the smaller portion of total starch in gluten-free pasta formulation, but it was still higher then semolina pasta.

The quality of a pasta is determined by its cooking properties as evaluated through optimal cooking time, swelling index, water absorption index, and cooking loss. The addition of egg white protein increased optimum cooking time, while soy protein isolate did not give significant differences either in 100% gluten-free banana pasta (Chapter 6) or in gluten-free pasta based on 75% banana flour and 25% cassava flour (Chapter 7). This showed that egg white protein formed a stronger structure that retard water penetration compared to soy protein isolate. Shorter optimum cooking time illustrated a weaker protein network that allowing a faster water penetration rate in the pasta structure (Phongthai et al., 2017). The inclusion of protein reduced the swelling index of gluten-free pasta (Chapter 6 & Chapter 7) due to a greater protein-starch network formation in the presence of sufficient protein in gluten-free pasta structure. A complex protein-starch network might inhibit the swelling and gelatinisation of starch granules (Desai et al., 2019). The water absorption index of gluten-free pasta increased by the increase of egg white protein addition but did not change with soy protein isolate inclusion. Phongthai et al. (2017) also found that egg white protein gave a better effect on improving water adsorption index compared to soy protein isolate in gluten-free pasta based on rice flour. The cooking loss of gluten-free pasta, which is the most noticeable parameter in the cooking quality of pasta, showed significant improvement with the increasing of protein incorporation. This is in agreement with other studies that found reducing cooking loss by the increasing of protein addition in gluten-free pasta (Larrosa et al., 2016; Sarawong et al., 2014a). Egg white protein reduced more cooking loss compared to soy protein isolate and met a minimum 8% cooking loss for industry-standard (Foschia et al., 2015a). A lower cooking loss determined a structure strengthening that prevents material leaching during cooking (Palavecino et al., 2017).

Protein inclusion improved the texture properties of gluten-free pasta based on banana flour. The incorporation of 25% cassava flour into gluten-free pasta showed similar texture characteristics. Egg white protein performed a better firmness and extensibility improvement compared to soy protein isolate in both gluten-free pasta formulation (Chapter 6 & Chapter 7). Bravo-Núñez et al. (2020) reported similar results in gluten-free pasta cake based on rice flour that showed egg white protein and other animal protein exhibited better textural properties compared to vegetable protein enrichment.

Chapter 8 and chapter 9 presented nutritional quality and digestibility assessment of gluten-free pasta based on banana flour and composite flour made from 75% banana flour and 25% cassava flour in incorporation with egg white protein and soy protein isolate. Soy protein had a positive effect on total phenolic content (TPC) and antioxidant activities of all gluten-free pasta formulation while egg white protein did not showed any differences. These due to high TPC and antioxidant content in soy protein isolate. Soy protein isolate has been reported to have TPC and antioxidant activities and has been used as a functional ingredient in many food applications (Chen et al., 2020; Lorenzo et al., 2018). Most of the gluten-free pasta formulation had higher TPC and antioxidant capacities compared to semolina pasta mainly because of higher antioxidant capacities of banana flour compared to semolina flour. A 25% cassava flour, which had the lowest TPC and antioxidant activities, gave significant effect in reducing these values in the gluten-free pasta products incorporated with egg white protein.

Starch digestibility of gluten-free pasta reduced by the addition of egg white protein and but did not change significantly by soy protein isolate (Chapter 8 and Chapter 9). Egg white protein had a more powerful effect in reducing glucose released then soy protein isolate indicated a more compact structure of the protein-starch network in gluten-free pasta that inhibits enzymatic reaction to digest starch. This effect was more significant in the gluten-free pasta with 25% cassava flour portion as this pasta formulation had higher glucose released compared to 100% banana pasta. A level of soy protein isolate addition (5% to 15%) did not give significant effect on starch digestibility might because of various optimum range of protein addition in reducing starch digestibility. Duta et al. (2019) found a similar result on different levels of oat protein inclusion (18% and 35%) that did not give any glycaemic index difference in gluten-free oat pasta.

The protein addition improved protein digestibility in gluten-free pasta based on banana and cassava flours (Chapter 8 and Chapter 9). Soy protein isolate gave a higher protein digestibility compared to egg white protein mainly because of a weaker pasta structure that led more protein to be digested by an enzymatic reaction. Laleg et al. (2016a) reported a protein network link level related to the protein digestibility of wheat pasta enriched with faba and egg protein.

Amino acid profiles of gluten-free pasta enhanced by the protein addition (Chapter 8 & Chapter 9). A minimum 5% protein inclusion altered amino acid composition of gluten-free pasta to be better than semolina pasta and met a daily amino acid consumption recommendation (FAO, 2013). Protein addition also improved protein digestibility corrected amino acid score (PDCAAS) of gluten-free pasta and better than semolina pasta. Egg white protein performed a better PDCAAS compared to soy protein isolate addition in gluten-free pasta. This showed egg white protein had a superior protein quality followed better cooking qualities, texture properties, and digestibilities characteristics.

Sensory evaluation of gluten-free pasta based on banana and cassava flours presents in chapter 9 shows types of protein addition had different consumer acceptances. Egg white protein inclusion accepted by consumer in a range neither dislike nor like to slightly like. Soy protein isolate incorporation in gluten-free banana-cassava pasta had slightly dislike to neither dislike nor like. Similar acceptances were observed by (Detchewa et al., 2016; Zandonadi et al., 2012). Purchase intent of gluten-free pasta enriched with egg white protein showed a potential market (46% to 49%) compared to 81% purchase of intent of semolina pasta.

10.3 Recommendation for future work

This study illustrated that banana and cassava flour alongside with protein sources addition improved pasta quality and nutritional properties that may contribute to exploring alternative materials and technologies in gluten-free pasta production development. To understand more clearly regarding the starch-protein network of egg white protein and soy protein isolate forming in the gluten-free pasta structure, scanning electron microscopy and protein network link measurements are necessary to be evaluated further. It also needs to conduct the study related to an interaction of starch-fibre, starch-protein, and starch-fibre-protein combinations that affecting starch digestibility and protein digestibility. A study with different banana flour and cassava flour portion with protein addition also recommended performing to give more comprehensive characteristics in gluten-free pasta characteristics based on these materials.

Appendix A

Pasta pictures

A.1 Semolina pasta and gluten-free pasta made of different blend of cassava flour and banana flour without any protein addition

100% banana pasta

100% Cassava pasta

75% Banana : 25% Cassava pasta

50% Banana : 50% Cassava pasta

25% Banana : 75% Cassava pasta

A.2 Gluten-free pasta with protein addition (75% Banana: 25% Cassava pasta - cooked)

Semolina pasta

75% Banana : 25% Cassava

5 % 10 % 15 %

GF pasta with egg white protein addition

5 % 10 % 15 %

GF pasta with soy protein isolate addition

Appendix B

Sensory Evaluation

B.1 Lincoln University Human Ethics Committee (HEC) approval letter

Research Management Office

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PO Box 85084, Lincoln University
Lincoln 7647, Christchurch
New Zealand
www.lincoln.ac.nz

12 March 2020

Application No: 2020-10

Title: Sensory evaluation for gluten-free pasta

Applicant: A Rachman

The Lincoln University Human Ethics Committee has reviewed the above noted application. Thank you for your response to the questions which were forwarded to you on the Committee's behalf.

I am satisfied on the Committee's behalf that the issues of concern have been satisfactorily addressed. I am pleased to give final approval to your project.

Please note that this approval is valid for three years from today's date at which time you will need to reapply for renewal.

Once your field work has finished can you please advise the Human Ethics Secretary, Alison Hind, and confirm that you have complied with the terms of the ethical approval.

May I, on behalf of the Committee, wish you success in your research.

Yours sincerely

Grant Tavinor
Chair, Human Ethics Committee

PLEASE NOTE: The Human Ethics Committee has an audit process in place for applications. Please see 7.3 of the Human Ethics Committee Operating Procedures (ACHE) in the Lincoln University Policies and Procedures Manual for more information.

B.2 Sensory evaluation questionnaire

GLUTEN-FREE PASTA SENSORY ANALYSIS

This experiment is aiming to evaluate the sensory properties of gluten-free pasta made from banana and/or cassava flour. Pasta may contain wheat, banana, and/or cassava flours, egg white protein and or soy protein in different combination and proportion.

Participant Profile:

1. Gender
   - ☐ Male
   - ☐ Female
   - ☐ Indeterminate
2. Age
   - ☐ ≥ 20 years
   - ☐ 21-30 years
   - ☐ 31-40 years
   - ☐ 41-50 years
   - ☐ 51-60 years
   - ☐ ≤ 61 years
3. Pasta consumption frequent:
   - ☐ Every day
   - ☐ Two or three times a week
   - ☐ Sometimes in a week
   - ☐ Two or three times a month
   - ☐ Sometimes in a month
   - ☐ Occasionally

Sample code:

1. Please have a taste of the sample and tick the following question regarding each of the following attributes:

Attributes	extremely dislike	very dislike	moderately dislike	slightly dislike	neither like nor dislike	slightly like	moderately like	very like	Extremely like
Appearance									
Aroma									
Texture									
Flavour									
Bitterness									
After taste									
Overall									

2. How is the texture?

Too soft	Just about right	Too hard

3. How is the pasta flavour?

Too weak	Just about right	Too strong

4. Tick all that apply to the taste of the pasta

☐ salty	☐ sweet	☐ soft	☐ gravy	☐ soybean	☐ chewy
☐ bitter	☐ fruity	☐ pasty	☐ umami	☐ gummy	☐ savoury
☐ dry	☐ sour	☐ greasy	☐ bland	☐ moist	☐ roast

5. If this product were available in the market, would you like to purchase?

☐ Yes ☐ No

If there any comment that would you like to share to make further improvement?

Thank you for your participation.

B.3 Raw data of sensory evaluation results

Table A. 1 Demographics data

Subject code	Gender 1=Male 2=Female 3=Indeterminate	Age 1≥20 years 2=21-30 years 3=31-40 years 4=41-50 years 5=51-60 years 6=more than 61 years	Pasta consumption frequent 1=Everyday 2=Two or three times a week 3=Sometimes in a week 4=Two or three times a month 5=Sometimes in a month 6=Occasionally	Consumption explanation
1001	2	2	4	
1002	2	2	3	
1003	1	3	3	
1004	2	3	3	
1005	1	2	3	
1006	1	2	5	

Subject code	Gender	Age	Pasta consumption frequent	Consumption explanation
1007	1	3	6	It is not common in my country
1008	1	4	4	
1009	1	2	3	
1010	1	3	2	
1011	2	1	3	
1012	2	3	3	
1013	1	3	5	
1014	1	3	5	
1015	2	3	3	
1016	1	3	4	
1017	1	6	6	Not a pasta person
1018	1	5	4	
1019	2	2	4	
1020	2	3	5	
1021	1	2	4	
1022	2	3	5	
1023	1	2	5	
1024	2	2	6	Habit
1025	2	2	4	
1026	1	3	5	

Subject code	Gender	Age	Pasta consumption frequent	Consumption explanation
1027	1	1	3	
1028	1	3	3	
1029	2	2	2	
1030	2	2	3	
1032	1	3	5	
1033	1	3	6	Very rarely
1034	2	2	4	
1035	1	3	2	
1036	2	3	2	
1037	1	2	4	
1040	1	5	5	

Table A.2. Questionnaire results

Subject Code	Serving Position	Sample Identi-fier	Sample Name	Liking Grid: Pasta appearance 1=Dislike Extremely 2=Dislike Very Much 3=Dislike Moderately 4=Dislike Slightly 5=Neither Like nor Dislike 6=Like Slightly 7=Like Moderately 8=Like Very Much 9=Like Extremely	Liking Grid: Pasta aroma 1=Dislike Extremely 2=Dislike Very Much 3=Dislike Moderately 4=Dislike Slightly 5=Neither Like nor Dislike 6=Like Slightly 7=Like Moderately 8=Like Very Much 9=Like Extremely	Liking Grid: Pasta texture 1=Dislike Extremely 2=Dislike Very Much 3=Dislike Moderately 4=Dislike Slightly 5=Neither Like nor Dislike 6=Like Slightly 7=Like Moderately 8=Like Very Much 9=Like Extremely	Liking Grid: Pasta flavour 1=Dislike Extremely 2=Dislike Very Much 3=Dislike Moderately 4=Dislike Slightly 5=Neither Like nor Dislike 6=Like Slightly 7=Like Moderately 8=Like Very Much 9=Like Extremely	Liking Grid: Bitterness 1=Dislike Extremely 2=Dislike Very Much 3=Dislike Moderately 4=Dislike Slightly 5=Neither Like nor Dislike 6=Like Slightly 7=Like Moderately 8=Like Very Much 9=Like Extremely	Liking Grid: Pasta aftertaste 1=Dislike Extremely 2=Dislike Very Much 3=Dislike Moderately 4=Dislike Slightly 5=Neither Like nor Dislike 6=Like Slightly 7=Like Moderately 8=Like Very Much 9=Like Extremely	Liking Grid: Overall liking 1=Dislike Extremely 2=Dislike Very Much 3=Dislike Moderately 4=Dislike Slightly 5=Neither Like nor Dislike 6=Like Slightly 7=Like Moderately 8=Like Very Much 9=Like Extremely	Overall Texture 1=Too Soft 2=Just About Right 3=Too Hard	Overall Flavour 1=Too Weak 2=Just About Right 3=Too Strong
1001	**1**	**137**	**15% Egg white**	7	5	7	7	5	5	6	2	2
1002	**3**	**137**	**15% Egg white**	3	1	2	2	5	2	2	3	3
1003	**2**	**137**	**15% Egg white**	5	6	7	7	7	6	6	2	1
1004	**5**	**137**	**15% Egg white**	5	6	6	6	5	5	5	1	2
1005	**4**	**137**	**15% Egg white**	7	5	7	4	5	5	5	2	1

Subject Code	Serving Position	Sample Identi-fier	Sample Name	Liking Grid: Pasta appearance	Liking Grid: Pasta aroma	Liking Grid: Pasta texture	Liking Grid: Pasta flavour	Liking Grid: Bitterness	Liking Grid: Pasta aftertaste	Liking Grid: Overall liking	Overall Texture	Overall Flavour
1006	**2**	**137**	**15% Egg white**	5	7	4	7	6	5	6	1	2
1007	**1**	**137**	**15% Egg white**	6	7	6	7	5	6	6	2	2
1008	**3**	**137**	**15% Egg white**	7	6	7	6	5	5	6	2	3
1009	**5**	**137**	**15% Egg white**	6	6	5	6	7	6	7	2	2
1010	**4**	**137**	**15% Egg white**	4	3	7	5	4	4	5	2	2
1011	**4**	**137**	**15% Egg white**	5	5	5	5	5	3	4	2	2
1012	**5**	**137**	**15% Egg white**	9	8	9	8	5	8	8	2	2
1013	**2**	**137**	**15% Egg white**	5	5	6	5	5	5	5	2	1
1014	**1**	**137**	**15% Egg white**	4	7	7	7	8	7	7	3	2
1015	**3**	**137**	**15% Egg white**	3	5	6	7	5	7	6	2	3
1016	**5**	**137**	**15% Egg white**	7	8	6	8	7	8	8	3	2
1017	**2**	**137**	**15% Egg white**	5	5	5	4	5	5	5	2	1
1018	**4**	**137**	**15% Egg white**	6	4	7	7	7	7	7	3	2
1019	**1**	**137**	**15% Egg white**	5	6	7	7	5	5	7	2	3
1020	**3**	**137**	**15% Egg white**	7	7	7	7	7	7	7	2	2
1021	**2**	**137**	**15% Egg white**	5	3	6	5	7	4	7	2	2
1022	**1**	**137**	**15% Egg white**	3	7	7	6	8	8	7	2	2
1023	**5**	**137**	**15% Egg white**	5	5	7	8	5	8	8	2	2
1024	**4**	**137**	**15% Egg white**	5	6	4	5	4	4	4	3	1
1025	**3**	**137**	**15% Egg white**	1	3	3	3	3	2	4	1	3
1026	**5**	**137**	**15% Egg white**	5	7	6	6	5	6	6	2	2
1027	**1**	**137**	**15% Egg white**	8	4	7	4	3	3	4	2	3
1028	**3**	**137**	**15% Egg white**	8	6	7	8	7	8	8	2	2
1029	**4**	**137**	**15% Egg white**	6	5	5	4	5	4	5	2	3
1030	**2**	**137**	**15% Egg white**	5	5	5	6	5	6	6	2	2
1032	**1**	**137**	**15% Egg white**	7	6	6	7	6	6	6	2	2
1033	**4**	**137**	**15% Egg white**	7	6	5	4	5	4	5	2	2
1034	**3**	**137**	**15% Egg white**	4	5	4	4	5	5	4	3	1
1035	**5**	**137**	**15% Egg white**	6	5	6	3	4	4	4	3	1
1036	**2**	**137**	**15% Egg white**	7	4	6	3	4	7	5	2	2

Subject Code	Serving Position	Sample Identi-fier	Sample Name	Liking Grid: Pasta appearance	Liking Grid: Pasta aroma	Liking Grid: Pasta texture	Liking Grid: Pasta flavour	Liking Grid: Bitterness	Liking Grid: Pasta aftertaste	Liking Grid: Overall liking	Overall Texture	Overall Flavour
1037	**1**	**137**	**15% Egg white**	8	5	6	4	4	5	6	2	2
1040	**4**	**137**	**15% Egg white**	2	2	2	2	2	2	2	3	1
1001	**2**	**329**	**Semolina pasta**	8	7	7	7	5	5	7	2	2
1002	**1**	**329**	**Semolina pasta**	8	6	7	6	5	5	7	2	2
1003	**4**	**329**	**Semolina pasta**	9	7	8	8	8	8	8	2	2
1004	**3**	**329**	**Semolina pasta**	7	7	8	8	5	8	8	2	2
1005	**5**	**329**	**Semolina pasta**	8	5	7	7	6	6	7	2	2
1006	**1**	**329**	**Semolina pasta**	7	5	7	5	6	5	7	2	2
1007	**3**	**329**	**Semolina pasta**	7	8	8	8	5	8	8	2	2
1008	**5**	**329**	**Semolina pasta**	8	5	7	7	7	7	7	2	2
1009	**4**	**329**	**Semolina pasta**	8	7	8	8	9	7	9	2	2
1010	**2**	**329**	**Semolina pasta**	8	8	8	7	5	8	8	2	2
1011	**5**	**329**	**Semolina pasta**	7	5	5	5	6	6	6	2	2
1012	**3**	**329**	**Semolina pasta**	8	8	6	7	7	7	7	3	1
1013	**4**	**329**	**Semolina pasta**	6	6	6	5	5	6	6	2	2
1014	**2**	**329**	**Semolina pasta**	8	8	7	7	7	8	8	2	2
1015	**1**	**329**	**Semolina pasta**	5	6	7	5	8	7	7	1	2
1016	**4**	**329**	**Semolina pasta**	8	7	7	5	5	6	7	3	1
1017	**1**	**329**	**Semolina pasta**	5	5	5	3	5	5	4	2	1
1018	**2**	**329**	**Semolina pasta**	7	7	8	8	7	8	8	2	2
1019	**3**	**329**	**Semolina pasta**	8	5	8	5	7	8	8	2	2
1020	**5**	**329**	**Semolina pasta**	9	9	9	9	8	9	9	2	2
1021	**3**	**329**	**Semolina pasta**	8	7	5	8	8	9	8	2	2
1022	**5**	**329**	**Semolina pasta**	9	8	8	8	5	4	7	2	3
1023	**2**	**329**	**Semolina pasta**	9	9	6	5	8	7	8	2	2
1024	**1**	**329**	**Semolina pasta**	8	8	8	8	6	8	8	2	2
1025	**4**	**329**	**Semolina pasta**	9	9	9	9	9	9	9	2	2
1026	**1**	**329**	**Semolina pasta**	7	5	6	5	7	7	5	2	2
1027	**4**	**329**	**Semolina pasta**	7	5	6	7	5	7	8	2	2
1028	**2**	**329**	**Semolina pasta**	6	4	7	6	6	6	7	2	3

Subject Code	Serving Position	Sample Identi-fier	Sample Name	Liking Grid: Pasta appearance	Liking Grid: Pasta aroma	Liking Grid: Pasta texture	Liking Grid: Pasta flavour	Liking Grid: Bitterness	Liking Grid: Pasta aftertaste	Liking Grid: Overall liking	Overall Texture	Overall Flavour
1029	**3**	**329**	**Semolina pasta**	6	5	6	8	5	7	7	3	2
1030	**5**	**329**	**Semolina pasta**	6	5	5	4	6	5	6	2	1
1032	**2**	**329**	**Semolina pasta**	7	6	7	7	7	6	7	2	2
1033	**5**	**329**	**Semolina pasta**	3	5	6	6	5	5	6	2	1
1034	**1**	**329**	**Semolina pasta**	5	5	7	5	5	6	5	1	1
1035	**3**	**329**	**Semolina pasta**	6	7	4	6	6	6	6	3	2
1036	**1**	**329**	**Semolina pasta**	7	8	6	8	8	8	8	1	2
1037	**3**	**329**	**Semolina pasta**	7	5	6	7	6	5	7	2	2
1040	**2**	**329**	**Semolina pasta**	8	8	8	8	8	8	8	2	2
1001	**4**	**402**	**5% Soy protein**	6	6	6	5	6	5	5	1	2
1002	**2**	**402**	**5% Soy protein**	2	2	3	2	5	3	4	1	3
1003	**5**	**402**	**5% Soy protein**	4	5	6	5	5	4	5	2	1
1004	**1**	**402**	**5% Soy protein**	5	6	5	4	5	3	4	1	2
1005	**3**	**402**	**5% Soy protein**	2	5	1	5	6	5	5	1	1
1006	**3**	**402**	**5% Soy protein**	3	5	5	5	5	5	4	2	1
1007	**5**	**402**	**5% Soy protein**	3	5	6	5	5	5	4	1	1
1008	**4**	**402**	**5% Soy protein**	5	5	5	6	5	5	5	1	2
1009	**2**	**402**	**5% Soy protein**	5	6	4	7	8	8	7	2	2
1010	**1**	**402**	**5% Soy protein**	2	2	5	5	5	5	3	2	1
1011	**2**	**402**	**5% Soy protein**	5	5	4	3	3	4	3	2	2
1012	**4**	**402**	**5% Soy protein**	4	5	6	5	4	5	5	3	1
1013	**1**	**402**	**5% Soy protein**	6	4	6	6	5	5	5	2	1
1014	**3**	**402**	**5% Soy protein**	5	4	4	5	5	5	4	2	3
1015	**5**	**402**	**5% Soy protein**	4	3	4	5	5	6	5	1	3
1016	**3**	**402**	**5% Soy protein**	2	7	4	7	6	8	7	2	2
1017	**4**	**402**	**5% Soy protein**	5	5	5	5	4	4	4	2	2
1018	**5**	**402**	**5% Soy protein**	1	3	2	2	3	2	2	1	1
1019	**2**	**402**	**5% Soy protein**	4	4	4	5	5	3	5	1	3
1020	**1**	**402**	**5% Soy protein**	4	4	4	4	4	4	4	1	3
1021	**1**	**402**	**5% Soy protein**	5	4	8	8	8	9	7	2	1

Subject Code	Serving Position	Sample Identi-fier	Sample Name	Liking Grid: Pasta appearance	Liking Grid: Pasta aroma	Liking Grid: Pasta texture	Liking Grid: Pasta flavour	Liking Grid: Bitterness	Liking Grid: Pasta aftertaste	Liking Grid: Overall liking	Overall Texture	Overall Flavour
1022	**3**	**402**	**5% Soy protein**	1	3	4	7	6	7	6	3	2
1023	**4**	**402**	**5% Soy protein**	5	7	6	5	5	5	7	1	1
1024	**2**	**402**	**5% Soy protein**	6	8	6	6	3	6	5	2	2
1025	**5**	**402**	**5% Soy protein**	9	9	9	9	9	9	9	3	1
1026	**3**	**402**	**5% Soy protein**	5	6	7	5	6	6	6	2	2
1027	**2**	**402**	**5% Soy protein**	2	5	2	5	5	4	3	1	1
1028	**1**	**402**	**5% Soy protein**	3	5	4	7	7	7	6	2	2
1029	**5**	**402**	**5% Soy protein**	4	3	4	6	5	5	6	1	2
1030	**4**	**402**	**5% Soy protein**	4	5	4	4	6	5	4	1	1
1032	**5**	**402**	**5% Soy protein**	5	4	4	5	5	4	5	1	3
1033	**1**	**402**	**5% Soy protein**	5	6	4	6	5	4	5	2	2
1034	**4**	**402**	**5% Soy protein**	5	4	6	4	5	4	4	1	1
1035	**2**	**402**	**5% Soy protein**	4	3	2	4	5	6	4	1	2
1036	**5**	**402**	**5% Soy protein**	2	3	1	5	3	2	3	1	1
1037	**4**	**402**	**5% Soy protein**	3	4	2	3	3	3	3	1	1
1040	**3**	**402**	**5% Soy protein**	2	2	2	2	1	2	2	1	1
1001	**5**	**614**	**15% Soy protein**	5	6	5	6	5	5	5	1	2
1002	**4**	**614**	**15% Soy protein**	4	3	4	3	5	4	4	2	2
1003	**3**	**614**	**15% Soy protein**	4	5	6	6	6	5	6	2	1
1004	**2**	**614**	**15% Soy protein**	4	6	3	5	5	3	3	1	1
1005	**1**	**614**	**15% Soy protein**	3	6	5	2	4	5	3	1	1
1006	**5**	**614**	**15% Soy protein**	4	5	4	4	5	4	4	1	1
1007	**4**	**614**	**15% Soy protein**	4	5	4	4	5	6	5	1	2
1008	**2**	**614**	**15% Soy protein**	5	6	6	7	5	5	6	1	3
1009	**1**	**614**	**15% Soy protein**	5	5	3	6	6	3	4	1	2
1010	**3**	**614**	**15% Soy protein**	2	1	6	3	3	2	2	2	3
1011	**3**	**614**	**15% Soy protein**	5	5	5	6	6	6	6	2	2
1012	**1**	**614**	**15% Soy protein**	6	6	6	6	6	7	6	2	2
1013	**5**	**614**	**15% Soy protein**	5	5	5	5	5	5	5	3	1
1014	**4**	**614**	**15% Soy protein**	5	4	2	2	2	2	2	3	3

Subject Code	Serving Position	Sample Identi-fier	Sample Name	Liking Grid: Pasta appearance	Liking Grid: Pasta aroma	Liking Grid: Pasta texture	Liking Grid: Pasta flavour	Liking Grid: Bitterness	Liking Grid: Pasta aftertaste	Liking Grid: Overall liking	Overall Texture	Overall Flavour
1015	**2**	**614**	**15% Soy protein**	4	5	7	6	7	8	7	2	2
1016	**2**	**614**	**15% Soy protein**	3	7	7	6	5	8	7	2	2
1017	**3**	**614**	**15% Soy protein**	5	5	5	4	5	4	4	2	1
1018	**1**	**614**	**15% Soy protein**	2	3	3	5	5	5	5	2	1
1019	**5**	**614**	**15% Soy protein**	3	4	1	5	1	4	4	3	3
1020	**4**	**614**	**15% Soy protein**	6	8	6	8	8	8	8	2	2
1021	**4**	**614**	**15% Soy protein**	3	4	7	5	3	4	5	2	2
1022	**2**	**614**	**15% Soy protein**	2	7	7	6	5	5	5	1	2
1023	**3**	**614**	**15% Soy protein**	5	6	4	5	5	5	5	1	2
1024	**5**	**614**	**15% Soy protein**	4	6	5	2	5	2	3	1	2
1025	**1**	**614**	**15% Soy protein**	3	5	9	9	7	9	7	2	2
1026	**4**	**614**	**15% Soy protein**	4	5	5	4	6	5	5	2	2
1027	**3**	**614**	**15% Soy protein**	3	3	1	2	3	2	1	1	3
1028	**5**	**614**	**15% Soy protein**	3	4	4	3	4	5	4	1	3
1029	**2**	**614**	**15% Soy protein**	4	6	6	6	5	6	6	2	3
1030	**1**	**614**	**15% Soy protein**	4	5	3	3	5	4	4	1	2
1032	**4**	**614**	**15% Soy protein**	6	5	5	6	5	5	5	1	3
1033	**3**	**614**	**15% Soy protein**	4	5	6	6	5	6	6	2	2
1034	**2**	**614**	**15% Soy protein**	6	6	5	4	5	4	4	1	3
1035	**1**	**614**	**15% Soy protein**	4	5	4	4	4	4	4	3	2
1036	**3**	**614**	**15% Soy protein**	7	6	5	6	5	7	5	2	2
1037	**5**	**614**	**15% Soy protein**	4	5	4	4	4	4	4	1	1
1040	**1**	**614**	**15% Soy protein**	3	4	5	6	7	6	6	2	3
1001	**3**	**875**	**5% Egg white**	5	4	6	6	6	5	5	2	2
1002	**5**	**875**	**5% Egg white**	3	4	5	4	5	4	4	2	2
1003	**1**	**875**	**5% Egg white**	4	7	7	7	7	7	7	2	1
1004	**4**	**875**	**5% Egg white**	4	5	3	4	5	4	4	1	1
1005	**2**	**875**	**5% Egg white**	6	5	8	5	5	5	6	2	2
1006	**4**	**875**	**5% Egg white**	5	6	4	6	5	6	5	1	2
1007	**2**	**875**	**5% Egg white**	7	6	7	7	5	5	8	2	2

Subject Code	Serving Position	Sample Identi-fier	Sample Name	Liking Grid: Pasta appearance	Liking Grid: Pasta aroma	Liking Grid: Pasta texture	Liking Grid: Pasta flavour	Liking Grid: Bitterness	Liking Grid: Pasta aftertaste	Liking Grid: Overall liking	Overall Texture	Overall Flavour
1008	**1**	**875**	**5% Egg white**	7	6	7	7	6	6	7	2	2
1009	**3**	**875**	**5% Egg white**	6	6	6	7	8	8	8	2	2
1010	**5**	**875**	**5% Egg white**	5	2	8	7	5	4	6	2	2
1011	**1**	**875**	**5% Egg white**	5	5	5	4	4	5	4	2	2
1012	**2**	**875**	**5% Egg white**	8	6	8	7	8	7	7	2	2
1013	**3**	**875**	**5% Egg white**	6	5	5	5	5	6	5	2	1
1014	**5**	**875**	**5% Egg white**	5	5	6	5	4	4	5	2	2
1015	**4**	**875**	**5% Egg white**	5	6	7	6	7	8	7	2	2
1016	**1**	**875**	**5% Egg white**	2	5	7	7	8	7	6	2	2
1017	**5**	**875**	**5% Egg white**	4	5	5	5	5	5	5	2	2
1018	**3**	**875**	**5% Egg white**	7	4	7	7	7	7	7	1	2
1019	**4**	**875**	**5% Egg white**	7	7	7	8	6	6	7	2	2
1020	**2**	**875**	**5% Egg white**	7	7	7	6	7	7	7	2	2
1021	**5**	**875**	**5% Egg white**	7	6	7	7	6	3	4	3	3
1022	**4**	**875**	**5% Egg white**	5	3	3	3	5	4	3	3	3
1023	**1**	**875**	**5% Egg white**	5	4	7	2	2	2	3	2	3
1024	**3**	**875**	**5% Egg white**	7	7	6	3	3	3	4	1	2
1025	**2**	**875**	**5% Egg white**	1	3	4	1	1	1	1	2	3
1026	**2**	**875**	**5% Egg white**	5	4	6	7	5	6	7	2	1
1027	**5**	**875**	**5% Egg white**	6	4	5	4	4	4	4	2	3
1028	**4**	**875**	**5% Egg white**	8	7	8	7	7	5	8	2	3
1029	**1**	**875**	**5% Egg white**	7	6	5	6	6	4	6	3	3
1030	**3**	**875**	**5% Egg white**	4	3	3	3	5	5	5	3	2
1032	**3**	**875**	**5% Egg white**	6	5	5	6	6	6	6	2	3
1033	**2**	**875**	**5% Egg white**	7	5	8	6	6	6	6	2	2
1034	**5**	**875**	**5% Egg white**	6	3	6	5	5	4	5	2	2
1035	**4**	**875**	**5% Egg white**	6	4	8	5	6	7	7	2	2
1036	**4**	**875**	**5% Egg white**	8	8	6	7	5	8	6	1	2
1037	**2**	**875**	**5% Egg white**	7	4	4	4	4	5	4	1	1
1040	**5**	**875**	**5% Egg white**	4	4	4	4	4	4	4	2	1

Table A3. Questionnaire results continue

Subject Code	Serving Position	Sample Identifier	Sample Name	Thick all that apply to the taste of the pasta (multi-select 0=not apply, 1=apply):									
				Salty	Bitter	Dry	Sweet	Fruity	Sour	Soft	Pasty	Greasy	Gravy
1001	**1**	**137**	**15% Egg white**	0	0	0	0	0	0	0	0	0	0
1002	**3**	**137**	**15% Egg white**	0	0	1	0	0	0	0	0	0	0
1003	**2**	**137**	**15% Egg white**	0	0	0	0	0	0	1	0	1	0
1004	**5**	**137**	**15% Egg white**	0	0	0	0	1	0	1	0	0	0
1005	**4**	**137**	**15% Egg white**	0	0	1	0	0	0	0	0	0	0
1006	**2**	**137**	**15% Egg white**	0	0	1	0	0	0	1	1	0	0
1007	**1**	**137**	**15% Egg white**	0	0	1	0	0	0	0	0	0	1
1008	**3**	**137**	**15% Egg white**	0	1	0	0	0	0	0	0	0	0
1009	**5**	**137**	**15% Egg white**	0	0	0	1	0	0	0	0	0	0
1010	**4**	**137**	**15% Egg white**	0	0	0	0	0	0	1	0	0	0
1011	**4**	**137**	**15% Egg white**	0	0	1	0	0	0	0	0	0	0
1012	**5**	**137**	**15% Egg white**	0	0	0	0	0	0	0	1	0	0
1013	**2**	**137**	**15% Egg white**	0	0	0	0	0	0	0	0	0	0
1014	**1**	**137**	**15% Egg white**	0	0	1	1	0	0	1	0	0	0
1015	**3**	**137**	**15% Egg white**	0	0	0	0	1	0	0	0	1	0
1016	**5**	**137**	**15% Egg white**	0	0	1	0	1	0	0	0	0	0
1017	**2**	**137**	**15% Egg white**	0	0	0	0	0	0	1	0	0	0
1018	**4**	**137**	**15% Egg white**	0	0	1	0	0	0	0	1	0	0
1019	**1**	**137**	**15% Egg white**	0	0	1	0	0	0	1	0	0	0
1020	**3**	**137**	**15% Egg white**	0	0	0	1	0	0	0	0	0	0
1021	**2**	**137**	**15% Egg white**	0	0	1	0	0	0	0	0	0	0
1022	**1**	**137**	**15% Egg white**	0	0	0	0	0	0	1	0	0	0
1023	**5**	**137**	**15% Egg white**	0	0	1	0	0	0	0	1	0	0
1024	**4**	**137**	**15% Egg white**	0	0	1	0	0	0	0	0	0	0
1025	**3**	**137**	**15% Egg white**	1	1	0	0	0	1	0	0	1	0
1026	**5**	**137**	**15% Egg white**	0	0	0	1	0	0	0	0	0	0
1027	**1**	**137**	**15% Egg white**	0	1	0	0	0	0	1	0	0	0
1028	**3**	**137**	**15% Egg white**	0	0	0	0	0	0	0	0	0	0
1029	**4**	**137**	**15% Egg white**	0	0	0	0	0	0	0	1	0	0
1030	**2**	**137**	**15% Egg white**	0	0	0	0	0	0	0	1	0	0

Subject Code	Serving Position	Sample Identifier	Sample Name	Thick all that apply to the taste of the pasta (multi-select 0=not apply, 1=apply):									
				Salty	Bitter	Dry	Sweet	Fruity	Sour	Soft	Pasty	Greasy	Gravy
1032	**1**	**137**	**15% Egg white**	0	0	0	0	0	0	0	0	1	0
1033	**4**	**137**	**15% Egg white**	1	0	0	0	0	0	0	0	0	0
1034	**3**	**137**	**15% Egg white**	0	0	0	0	0	0	0	0	0	0
1035	**5**	**137**	**15% Egg white**	0	0	1	0	0	1	0	0	0	0
1036	**2**	**137**	**15% Egg white**	0	0	1	0	0	0	0	0	0	0
1037	**1**	**137**	**15% Egg white**	0	0	1	0	0	0	0	0	0	0
1040	**4**	**137**	**15% Egg white**	0	1	1	0	0	0	0	0	0	0
1001	**2**	**329**	**Semolina pasta**	0	0	0	0	0	0	0	1	0	0
1002	**1**	**329**	**Semolina pasta**	0	0	0	0	0	0	0	0	0	0
1003	**4**	**329**	**Semolina pasta**	1	0	0	0	0	0	1	0	1	0
1004	**3**	**329**	**Semolina pasta**	0	0	0	0	0	0	0	0	0	0
1005	**5**	**329**	**Semolina pasta**	0	0	0	0	0	0	0	0	0	0
1006	**1**	**329**	**Semolina pasta**	0	0	0	0	0	0	1	0	0	0
1007	**3**	**329**	**Semolina pasta**	0	0	0	1	1	0	0	0	0	0
1008	**5**	**329**	**Semolina pasta**	0	0	0	0	0	0	0	1	0	0
1009	**4**	**329**	**Semolina pasta**	0	0	0	1	0	0	0	0	0	0
1010	**2**	**329**	**Semolina pasta**	0	0	0	0	0	0	0	0	0	0
1011	**5**	**329**	**Semolina pasta**	0	0	1	0	0	0	0	0	0	0
1012	**3**	**329**	**Semolina pasta**	0	0	1	0	0	0	0	0	0	0
1013	**4**	**329**	**Semolina pasta**	0	0	0	0	0	0	1	0	0	0
1014	**2**	**329**	**Semolina pasta**	0	0	0	1	0	0	1	0	0	0
1015	**1**	**329**	**Semolina pasta**	0	0	0	0	0	0	1	0	0	0
1016	**4**	**329**	**Semolina pasta**	0	0	1	0	0	0	0	0	0	0
1017	**1**	**329**	**Semolina pasta**	0	0	0	0	0	0	1	0	0	0
1018	**2**	**329**	**Semolina pasta**	0	0	0	0	0	0	0	0	0	0
1019	**3**	**329**	**Semolina pasta**	0	0	0	1	0	0	1	0	0	0
1020	**5**	**329**	**Semolina pasta**	1	0	0	1	0	1	0	0	1	0
1021	**3**	**329**	**Semolina pasta**	0	0	0	1	0	0	0	0	0	0
1022	**5**	**329**	**Semolina pasta**	0	1	0	0	0	0	1	1	0	0
1023	**2**	**329**	**Semolina pasta**	0	0	0	0	0	0	0	0	0	0
1024	**1**	**329**	**Semolina pasta**	0	0	0	0	0	0	1	0	0	0
1025	**4**	**329**	**Semolina pasta**	0	0	0	1	0	0	1	1	0	0

Subject Code	Serving Position	Sample Identifier	Sample Name	Thick all that apply to the taste of the pasta (multi-select 0=not apply, 1=apply):									
				Salty	Bitter	Dry	Sweet	Fruity	Sour	Soft	Pasty	Greasy	Gravy
1026	**1**	**329**	**Semolina pasta**	0	0	0	0	0	0	0	0	0	0
1027	**4**	**329**	**Semolina pasta**	0	0	0	0	0	0	1	0	0	0
1028	**2**	**329**	**Semolina pasta**	0	1	0	0	0	0	0	0	0	0
1029	**3**	**329**	**Semolina pasta**	0	0	0	0	0	0	0	0	0	0
1030	**5**	**329**	**Semolina pasta**	0	0	1	0	0	0	0	0	0	0
1032	**2**	**329**	**Semolina pasta**	0	0	0	0	0	0	0	0	0	0
1033	**5**	**329**	**Semolina pasta**	0	0	1	0	0	0	0	0	0	0
1034	**1**	**329**	**Semolina pasta**	0	0	0	0	0	0	1	1	0	0
1035	**3**	**329**	**Semolina pasta**	0	0	1	1	0	0	0	0	0	0
1036	**1**	**329**	**Semolina pasta**	0	0	0	0	0	0	0	1	0	0
1037	**3**	**329**	**Semolina pasta**	0	0	0	0	0	0	1	0	0	0
1040	**2**	**329**	**Semolina pasta**	0	0	0	0	0	0	1	1	0	0
1001	**4**	**402**	**5% Soy protein**	0	0	0	0	0	0	1	0	0	0
1002	**2**	**402**	**5% Soy protein**	0	0	0	0	0	0	1	0	0	0
1003	**5**	**402**	**5% Soy protein**	0	0	0	0	0	0	1	1	1	0
1004	**1**	**402**	**5% Soy protein**	0	0	0	0	0	0	1	0	0	0
1005	**3**	**402**	**5% Soy protein**	0	0	0	0	0	0	1	0	0	0
1006	**3**	**402**	**5% Soy protein**	0	0	0	0	0	0	1	0	0	0
1007	**5**	**402**	**5% Soy protein**	0	0	0	0	0	0	0	0	0	0
1008	**4**	**402**	**5% Soy protein**	0	0	0	0	0	0	0	0	0	0
1009	**2**	**402**	**5% Soy protein**	0	0	0	1	0	0	1	0	0	0
1010	**1**	**402**	**5% Soy protein**	0	0	1	0	0	0	1	0	0	0
1011	**2**	**402**	**5% Soy protein**	0	1	1	0	0	0	0	0	0	0
1012	**4**	**402**	**5% Soy protein**	0	1	1	0	0	0	0	0	0	0
1013	**1**	**402**	**5% Soy protein**	0	0	1	0	0	0	0	0	0	0
1014	**3**	**402**	**5% Soy protein**	0	0	0	1	1	0	0	0	0	0
1015	**5**	**402**	**5% Soy protein**	0	0	0	0	0	0	0	1	1	1
1016	**3**	**402**	**5% Soy protein**	0	0	1	0	0	0	0	0	0	0
1017	**4**	**402**	**5% Soy protein**	0	0	0	0	0	0	1	0	0	0
1018	**5**	**402**	**5% Soy protein**	0	1	0	0	0	0	1	0	0	0
1019	**2**	**402**	**5% Soy protein**	0	1	0	0	0	0	1	0	0	0
1020	**1**	**402**	**5% Soy protein**	0	0	0	1	0	0	0	0	1	0

Subject Code	Serving Position	Sample Identifier	Sample Name	Thick all that apply to the taste of the pasta (multi-select 0=not apply, 1=apply):									
				Salty	Bitter	Dry	Sweet	Fruity	Sour	Soft	Pasty	Greasy	Gravy
1021	**1**	**402**	**5% Soy protein**	0	0	1	0	0	0	1	0	0	0
1022	**3**	**402**	**5% Soy protein**	0	0	0	0	0	0	0	0	0	0
1023	**4**	**402**	**5% Soy protein**	0	1	0	0	0	0	1	0	0	0
1024	**2**	**402**	**5% Soy protein**	0	1	1	0	0	0	1	0	0	0
1025	**5**	**402**	**5% Soy protein**	0	0	0	0	0	0	0	1	0	0
1026	**3**	**402**	**5% Soy protein**	0	0	0	0	0	0	1	0	0	0
1027	**2**	**402**	**5% Soy protein**	0	0	0	0	0	0	0	1	0	0
1028	**1**	**402**	**5% Soy protein**	0	0	0	0	0	0	0	0	0	1
1029	**5**	**402**	**5% Soy protein**	0	0	0	0	0	0	1	0	0	0
1030	**4**	**402**	**5% Soy protein**	0	0	0	0	0	0	1	0	0	0
1032	**5**	**402**	**5% Soy protein**	0	0	0	0	0	0	1	0	0	0
1033	**1**	**402**	**5% Soy protein**	0	0	1	0	0	0	0	0	0	0
1034	**4**	**402**	**5% Soy protein**	0	0	0	0	0	1	0	0	0	0
1035	**2**	**402**	**5% Soy protein**	0	1	0	1	0	0	1	1	0	0
1036	**5**	**402**	**5% Soy protein**	0	0	0	0	0	0	1	1	0	0
1037	**4**	**402**	**5% Soy protein**	0	0	0	0	0	0	1	0	0	0
1040	**3**	**402**	**5% Soy protein**	0	1	0	0	0	0	1	1	0	0
1001	**5**	**614**	**15% Soy protein**	0	0	0	0	0	0	1	1	0	0
1002	**4**	**614**	**15% Soy protein**	0	0	0	1	0	0	0	0	0	0
1003	**3**	**614**	**15% Soy protein**	0	0	0	0	0	0	1	0	0	0
1004	**2**	**614**	**15% Soy protein**	0	0	0	0	0	0	1	1	0	0
1005	**1**	**614**	**15% Soy protein**	0	1	0	0	0	0	0	1	0	0
1006	**5**	**614**	**15% Soy protein**	0	0	0	0	0	0	0	0	0	0
1007	**4**	**614**	**15% Soy protein**	0	0	0	0	0	0	0	1	0	0
1008	**2**	**614**	**15% Soy protein**	0	1	0	0	0	0	0	0	1	1
1009	**1**	**614**	**15% Soy protein**	0	0	0	1	0	0	1	1	0	0
1010	**3**	**614**	**15% Soy protein**	0	1	1	0	0	0	0	1	0	0
1011	**3**	**614**	**15% Soy protein**	0	0	1	0	0	0	0	0	0	0
1012	**1**	**614**	**15% Soy protein**	0	0	0	0	0	0	0	1	0	0
1013	**5**	**614**	**15% Soy protein**	0	0	1	0	0	0	0	0	0	0
1014	**4**	**614**	**15% Soy protein**	0	0	1	0	1	0	0	0	1	0
1015	**2**	**614**	**15% Soy protein**	0	0	1	0	0	0	0	1	0	0

Subject Code	Serving Position	Sample Identifier	Sample Name	Thick all that apply to the taste of the pasta (multi-select 0=not apply, 1=apply):									
				Salty	Bitter	Dry	Sweet	Fruity	Sour	Soft	Pasty	Greasy	Gravy
1016	**2**	**614**	**15% Soy protein**	0	0	0	0	1	0	1	0	0	0
1017	**3**	**614**	**15% Soy protein**	0	0	0	0	0	0	0	0	0	0
1018	**1**	**614**	**15% Soy protein**	0	0	0	0	0	0	1	0	0	0
1019	**5**	**614**	**15% Soy protein**	0	1	1	0	0	0	0	0	0	0
1020	**4**	**614**	**15% Soy protein**	0	0	0	1	0	1	0	0	0	0
1021	**4**	**614**	**15% Soy protein**	0	1	1	0	0	0	0	0	0	0
1022	**2**	**614**	**15% Soy protein**	0	0	0	0	0	0	0	1	0	0
1023	**3**	**614**	**15% Soy protein**	0	1	1	0	0	0	1	0	0	0
1024	**5**	**614**	**15% Soy protein**	0	1	1	0	0	0	0	0	0	0
1025	**1**	**614**	**15% Soy protein**	0	0	1	0	0	0	1	1	0	0
1026	**4**	**614**	**15% Soy protein**	0	0	1	0	0	0	0	0	0	0
1027	**3**	**614**	**15% Soy protein**	0	1	0	0	0	0	1	1	0	0
1028	**5**	**614**	**15% Soy protein**	0	1	0	0	0	0	0	0	0	1
1029	**2**	**614**	**15% Soy protein**	0	0	0	0	0	0	1	1	0	0
1030	**1**	**614**	**15% Soy protein**	0	0	0	0	0	0	1	0	0	0
1032	**4**	**614**	**15% Soy protein**	0	0	0	0	0	0	1	0	0	0
1033	**3**	**614**	**15% Soy protein**	1	0	1	0	0	0	1	0	0	0
1034	**2**	**614**	**15% Soy protein**	0	1	1	0	0	0	0	0	0	0
1035	**1**	**614**	**15% Soy protein**	0	1	0	0	0	0	0	1	0	0
1036	**3**	**614**	**15% Soy protein**	0	0	0	0	0	0	0	1	0	0
1037	**5**	**614**	**15% Soy protein**	0	0	0	0	0	0	1	0	0	0
1040	**1**	**614**	**15% Soy protein**	0	0	0	0	0	0	0	0	0	0
1001	**3**	**875**	**5% Egg white**	0	0	0	0	0	0	1	0	0	0
1002	**5**	**875**	**5% Egg white**	0	0	0	1	0	0	0	0	0	0
1003	**1**	**875**	**5% Egg white**	0	0	0	0	0	0	1	0	1	0
1004	**4**	**875**	**5% Egg white**	0	0	0	0	0	0	1	0	0	0
1005	**2**	**875**	**5% Egg white**	0	0	0	0	0	0	1	0	0	0
1006	**4**	**875**	**5% Egg white**	0	0	0	0	0	0	0	1	0	0
1007	**2**	**875**	**5% Egg white**	0	0	0	1	1	0	0	0	0	0
1008	**1**	**875**	**5% Egg white**	0	0	0	0	0	0	0	0	0	0
1009	**3**	**875**	**5% Egg white**	0	0	0	1	0	0	0	0	0	0
1010	**5**	**875**	**5% Egg white**	0	0	0	0	0	0	0	0	0	0

Subject Code	Serving Position	Sample Identifier	Sample Name	Thick all that apply to the taste of the pasta (multi-select 0=not apply, 1=apply):									
				Salty	Bitter	Dry	Sweet	Fruity	Sour	Soft	Pasty	Greasy	Gravy
1011	**1**	**875**	**5% Egg white**	0	0	0	0	0	0	0	0	0	0
1012	**2**	**875**	**5% Egg white**	0	0	0	0	0	0	0	1	0	0
1013	**3**	**875**	**5% Egg white**	0	0	0	0	0	0	0	0	0	0
1014	**5**	**875**	**5% Egg white**	0	0	0	1	1	0	1	0	0	0
1015	**4**	**875**	**5% Egg white**	0	0	0	0	0	0	1	0	0	0
1016	**1**	**875**	**5% Egg white**	0	0	0	0	0	0	1	0	0	0
1017	**5**	**875**	**5% Egg white**	0	0	1	0	0	0	0	0	0	0
1018	**3**	**875**	**5% Egg white**	0	0	0	1	0	1	1	0	0	0
1019	**4**	**875**	**5% Egg white**	0	0	0	1	0	0	1	0	0	0
1020	**2**	**875**	**5% Egg white**	0	0	0	1	0	0	0	0	1	1
1021	**5**	**875**	**5% Egg white**	0	0	1	0	0	0	0	0	0	0
1022	**4**	**875**	**5% Egg white**	0	0	0	0	0	0	0	0	0	0
1023	**1**	**875**	**5% Egg white**	0	1	0	0	0	0	1	0	0	0
1024	**3**	**875**	**5% Egg white**	0	1	0	0	0	0	1	0	0	0
1025	**2**	**875**	**5% Egg white**	0	1	0	0	0	1	1	1	0	0
1026	**2**	**875**	**5% Egg white**	0	0	0	0	0	0	0	0	0	0
1027	**5**	**875**	**5% Egg white**	0	1	0	0	0	0	1	0	0	0
1028	**4**	**875**	**5% Egg white**	0	1	0	0	0	0	0	0	0	0
1029	**1**	**875**	**5% Egg white**	0	0	0	0	0	0	0	1	0	0
1030	**3**	**875**	**5% Egg white**	0	0	1	0	0	0	0	0	0	0
1032	**3**	**875**	**5% Egg white**	0	0	0	0	0	0	0	0	0	0
1033	**2**	**875**	**5% Egg white**	0	0	1	0	0	0	0	0	0	0
1034	**5**	**875**	**5% Egg white**	0	0	0	0	0	1	0	0	0	0
1035	**4**	**875**	**5% Egg white**	0	0	0	1	0	0	0	0	0	0
1036	**4**	**875**	**5% Egg white**	0	0	0	0	0	0	0	1	0	0
1037	**2**	**875**	**5% Egg white**	0	0	0	0	0	0	0	0	0	0
1040	**5**	**875**	**5% Egg white**	0	1	0	0	0	0	0	0	0	0

Table A.4 Questionnaire results Continue

Subject Code	Serving Position	Sample Identi-fier	Sample Name	Thick all that apply to the taste of the pasta (multi-select 0=not apply, 1=apply):								Would you purchase this product if it were available at a reasonable price where you normally shop? (1=No; 2=Yes)	If there any comment that would you like to share to make further improvement? (Text)
				Umami	Bland	Soybean	Gummy	Moist	Chewy	Savoury	Roast		
1001	**1**	**137**	**15% Egg white**	0	1	0	0	1	0	0	0	1	Good
1002	**3**	**137**	**15% Egg white**	0	0	0	0	0	0	0	0	1	R
1003	**2**	**137**	**15% Egg white**	0	0	0	0	0	1	0	0	1	Additional flavour requirements
1004	**5**	**137**	**15% Egg white**	0	0	1	0	0	0	0	0	1	No comment
1005	**4**	**137**	**15% Egg white**	0	1	0	0	0	0	0	0	1	Too dry.
1006	**2**	**137**	**15% Egg white**	0	1	0	0	0	0	0	0	2	It will be better if it is gummier.
1007	**1**	**137**	**15% Egg white**	0	0	0	0	1	0	0	0	2	No
1008	**3**	**137**	**15% Egg white**	0	1	0	0	0	0	1	0	1	Ok
1009	**5**	**137**	**15% Egg white**	0	0	0	0	0	1	0	0	2	When I try this product, there is taste like raw flour.
1010	**4**	**137**	**15% Egg white**	0	0	1	0	0	0	0	1	2	To be improved in colour and aftertaste
1011	**4**	**137**	**15% Egg white**	0	0	0	0	0	0	0	0	1	.
1012	**5**	**137**	**15% Egg white**	0	1	0	0	0	1	0	0	2	I preferred this sample. Improve its taste.
1013	**2**	**137**	**15% Egg white**	0	0	0	1	0	0	0	0	1	NOT
1014	**1**	**137**	**15% Egg white**	0	0	0	0	0	0	0	0	2	Make it softer
1015	**3**	**137**	**15% Egg white**	0	0	0	0	1	0	1	0	1	Too strong flavour
1016	**5**	**137**	**15% Egg white**	0	0	0	0	0	0	0	0	2	Should be a bit softer
1017	**2**	**137**	**15% Egg white**	0	0	0	0	0	0	0	0	1	Bland
1018	**4**	**137**	**15% Egg white**	0	0	0	0	0	1	0	0	1	Quite firm texture

Subject Code	Serving Position	Sample Identi-fier	Sample Name	Thick all that apply to the taste of the pasta (multi-select 0=not apply, 1=apply):								Would you purchase this product if it were available at a reasonable price where you normally shop? (1=No; 2=Yes)	If there any comment that would you like to share to make further improvement? (Text)
				Umami	Bland	Soybean	Gummy	Moist	Chewy	Savoury	Roast		
1019	**1**	**137**	**15% Egg white**	0	0	0	0	0	0	0	0	2	Maybe it would taste better if add some sauce with different flavour.
1020	**3**	**137**	**15% Egg white**	0	0	1	0	0	0	1	0	2	It is ok
1021	**2**	**137**	**15% Egg white**	0	0	0	1	0	1	0	0	2	I do not like that smell
1022	**1**	**137**	**15% Egg white**	0	0	0	0	1	0	0	0	2	No
1023	**5**	**137**	**15% Egg white**	0	0	1	0	0	1	0	0	2	No thanks
1024	**4**	**137**	**15% Egg white**	0	0	0	0	0	0	0	0	1	Texture and flavour
1025	**3**	**137**	**15% Egg white**	0	0	1	1	1	1	0	0	1	It is too sticky, does not look the best either
1026	**5**	**137**	**15% Egg white**	0	0	0	0	0	0	0	0	2	No
1027	**1**	**137**	**15% Egg white**	0	0	0	1	0	1	0	0	1	The after taste of the pasta was quite earthy and bitter
1028	**3**	**137**	**15% Egg white**	1	0	0	0	0	0	1	0	2	Like texture and flavour
1029	**4**	**137**	**15% Egg white**	0	0	0	0	0	0	0	0	1	Flavour too strong, too chewy, aftertaste lingers
1030	**2**	**137**	**15% Egg white**	0	0	0	1	0	1	0	0	2	They are not really sticky and taste way better than samples 614
1032	**1**	**137**	**15% Egg white**	0	1	0	0	0	0	0	0	2	.
1033	**4**	**137**	**15% Egg white**	0	0	1	0	0	0	1	1	1	No
1034	**3**	**137**	**15% Egg white**	0	1	0	0	0	0	0	0	1	The texture not smooth
1035	**5**	**137**	**15% Egg white**	0	0	0	0	0	0	0	0	1	Look good but taste not very nice. Better chewy needed
1036	**2**	**137**	**15% Egg white**	0	0	1	0	0	1	0	0	2	Seems harder

Subject Code	Serving Position	Sample Identi-fier	Sample Name	Thick all that apply to the taste of the pasta (multi-select 0=not apply, 1=apply):								Would you purchase this product if it were available at a reasonable price where you normally shop? (1=No; 2=Yes)	If there any comment that would you like to share to make further improvement? (Text)
				Umami	Bland	Soybean	Gummy	Moist	Chewy	Savoury	Roast		
1037	**1**	**137**	**15% Egg white**	0	0	0	0	0	1	0	1	1	Feels like ingredient does not combine
1040	**4**	**137**	**15% Egg white**	0	0	0	0	0	0	0	0	1	Not acceptable
1001	**2**	**329**	**Semolina pasta**	1	0	0	0	1	0	0	0	2	Looks good
1002	**1**	**329**	**Semolina pasta**	0	0	0	0	1	1	0	0	2	RT
1003	**4**	**329**	**Semolina pasta**	0	0	0	0	0	0	0	0	2	Just little bit of salt .... but it is good for me
1004	**3**	**329**	**Semolina pasta**	0	0	0	0	1	1	0	0	2	No comment
1005	**5**	**329**	**Semolina pasta**	0	0	0	1	0	1	1	0	2	This is the best one.
1006	**1**	**329**	**Semolina pasta**	0	0	0	0	1	1	0	0	2	Good enough to be sold in shop.
1007	**3**	**329**	**Semolina pasta**	1	0	0	0	0	0	0	0	2	No
1008	**5**	**329**	**Semolina pasta**	0	0	0	0	0	1	0	0	2	Good
1009	**4**	**329**	**Semolina pasta**	1	0	0	0	0	1	0	0	2	If the colour is closer to common pasta that will be better.
1010	**2**	**329**	**Semolina pasta**	0	0	0	0	1	0	1	0	2	No
1011	**5**	**329**	**Semolina pasta**	0	0	0	0	0	0	0	0	2	.
1012	**3**	**329**	**Semolina pasta**	0	1	0	0	0	1	0	0	2	Feel texture is hard
1013	**4**	**329**	**Semolina pasta**	0	0	0	0	0	0	0	0	1	NOT
1014	**2**	**329**	**Semolina pasta**	0	0	0	1	0	0	0	0	2	It is good
1015	**1**	**329**	**Semolina pasta**	0	0	0	0	1	0	0	0	2	Tasteless, maybe savoury taste suit for this colour
1016	**4**	**329**	**Semolina pasta**	0	0	0	0	0	0	0	0	1	Aroma and flavour are weak, hardly feel anything

Subject Code	Serving Position	Sample Identi-fier	Sample Name	Thick all that apply to the taste of the pasta (multi-select 0=not apply, 1=apply):								Would you purchase this product if it were available at a reasonable price where you normally shop? (1=No; 2=Yes)	If there any comment that would you like to share to make further improvement? (Text)
				Umami	Bland	Soybean	Gummy	Moist	Chewy	Savoury	Roast		
1017	**1**	**329**	**Semolina pasta**	0	1	0	0	0	0	0	0	1	Bland
1018	**2**	**329**	**Semolina pasta**	0	1	0	0	1	0	0	0	2	Well balanced
1019	**3**	**329**	**Semolina pasta**	0	0	0	0	1	0	0	0	2	No
1020	**5**	**329**	**Semolina pasta**	0	0	0	0	0	0	1	0	2	Very good like noodle
1021	**3**	**329**	**Semolina pasta**	0	0	0	0	0	1	0	0	2	Good enough
1022	**5**	**329**	**Semolina pasta**	0	0	0	0	0	0	0	0	2	after taste needs to be improved
1023	**2**	**329**	**Semolina pasta**	0	0	0	0	1	0	0	0	2	No thanks
1024	**1**	**329**	**Semolina pasta**	0	1	0	0	1	0	0	0	2	The flavour
1025	**4**	**329**	**Semolina pasta**	0	0	0	1	0	0	0	0	2	Nice
1026	**1**	**329**	**Semolina pasta**	0	1	0	0	0	0	0	0	1	No
1027	**4**	**329**	**Semolina pasta**	0	1	0	1	0	1	0	0	2	Appearance is nice, texture is very good and no after taste is good
1028	**2**	**329**	**Semolina pasta**	0	0	0	1	0	0	0	0	1	Strong flavour
1029	**3**	**329**	**Semolina pasta**	0	0	0	0	0	1	0	0	2	.
1030	**5**	**329**	**Semolina pasta**	0	0	0	1	0	1	0	0	2	It is chewy and gummy, but it doesn't have much flavour and kind of tasteless.
1032	**2**	**329**	**Semolina pasta**	0	0	0	1	0	0	0	0	2	,
1033	**5**	**329**	**Semolina pasta**	0	0	0	1	0	1	1	0	1	No
1034	**1**	**329**	**Semolina pasta**	0	1	0	0	0	0	0	0	2	Too soft Not much flavour
1035	**3**	**329**	**Semolina pasta**	0	0	0	0	0	1	0	0	2	Better texture
1036	**1**	**329**	**Semolina pasta**	0	0	0	1	1	0	0	0	1	Too sticky when touched

Subject Code	Serving Position	Sample Identi-fier	Sample Name	Thick all that apply to the taste of the pasta (multi-select 0=not apply, 1=apply):								Would you purchase this product if it were available at a reasonable price where you normally shop? (1=No; 2=Yes)	If there any comment that would you like to share to make further improvement? (Text)
				Umami	Bland	Soybean	Gummy	Moist	Chewy	Savoury	Roast		
1037	**3**	**329**	**Semolina pasta**	0	0	0	0	0	1	0	0	2	Bit more flavours
1040	**2**	**329**	**Semolina pasta**	0	0	0	0	0	1	0	0	2	It is acceptable
1001	**4**	**402**	**5% Soy protein**	0	0	0	0	1	0	0	1	1	N
1002	**2**	**402**	**5% Soy protein**	0	0	0	1	0	0	0	0	1	Rt
1003	**5**	**402**	**5% Soy protein**	0	0	0	0	0	0	0	0	1	Some modifications are required to enhance the sensory characteristics
1004	**1**	**402**	**5% Soy protein**	0	0	0	0	1	0	0	0	1	No comment
1005	**3**	**402**	**5% Soy protein**	0	1	1	1	0	1	0	0	1	Better than 614, but still not good.
1006	**3**	**402**	**5% Soy protein**	0	1	0	0	1	1	0	0	1	It is too moist
1007	**5**	**402**	**5% Soy protein**	0	1	0	0	1	0	0	0	2	No
1008	**4**	**402**	**5% Soy protein**	0	1	0	0	0	1	0	0	2	Yes
1009	**2**	**402**	**5% Soy protein**	0	0	0	0	0	1	1	0	2	Product 402 taste have a little bit like the raw flour mixing in it. The flavour and aftertaste I like it very much.
1010	**1**	**402**	**5% Soy protein**	0	0	0	0	0	0	0	0	1	Grainy texture
1011	**2**	**402**	**5% Soy protein**	0	0	0	0	0	0	0	0	1	.
1012	**4**	**402**	**5% Soy protein**	0	0	0	1	0	0	0	0	1	It is better if you can improve the flavour
1013	**1**	**402**	**5% Soy protein**	0	0	0	0	0	0	0	0	1	NOT
1014	**3**	**402**	**5% Soy protein**	0	0	0	0	0	0	0	0	1	Do not like the taste
1015	**5**	**402**	**5% Soy protein**	0	0	0	0	1	0	0	0	1	Too soft
1016	**3**	**402**	**5% Soy protein**	0	0	0	1	0	0	0	0	2	The size is a bit small

Subject Code	Serving Position	Sample Identi-fier	Sample Name	Thick all that apply to the taste of the pasta (multi-select 0=not apply, 1=apply):								Would you purchase this product if it were available at a reasonable price where you normally shop? (1=No; 2=Yes)	If there any comment that would you like to share to make further improvement? (Text)
				Umami	Bland	Soybean	Gummy	Moist	Chewy	Savoury	Roast		
1017	**4**	**402**	**5% Soy protein**	0	1	0	0	0	0	0	0	1	Bland
1018	**5**	**402**	**5% Soy protein**	0	0	0	1	1	1	0	0	1	Looks very yummy and like a gel
1019	**2**	**402**	**5% Soy protein**	0	0	0	0	1	0	0	0	1	Its Sticky somehow
1020	**1**	**402**	**5% Soy protein**	0	0	1	0	0	0	1	0	1	Too soft and strange taste
1021	**1**	**402**	**5% Soy protein**	0	0	0	0	0	1	0	0	2	Need stronger flavour
1022	**3**	**402**	**5% Soy protein**	0	0	0	1	0	1	0	0	1	Colour and texture need improvement
1023	**4**	**402**	**5% Soy protein**	0	0	0	0	1	0	0	0	1	No thanks
1024	**2**	**402**	**5% Soy protein**	0	1	0	0	0	0	0	0	1	The bitterness should be reduced
1025	**5**	**402**	**5% Soy protein**	0	1	1	0	0	0	0	0	2	It does not look like pasta, but it tastes good and has no after taste and does not smell bad
1026	**3**	**402**	**5% Soy protein**	0	0	0	0	0	0	0	0	2	No
1027	**2**	**402**	**5% Soy protein**	0	1	0	0	1	1	0	0	1	The texture is very glugs. The pasta is stuck together in a clump which is unappetizing
1028	**1**	**402**	**5% Soy protein**	0	0	0	0	0	0	0	0	1	More cooked
1029	**5**	**402**	**5% Soy protein**	0	0	0	0	0	0	0	0	1	Too soft too sticky but flavour ok, not too strong
1030	**4**	**402**	**5% Soy protein**	0	0	0	1	1	0	0	0	1	They stick together and does not look good. Some part is soft, and the other part is quite hard.

Subject Code	Serving Position	Sample Identi-fier	Sample Name	Thick all that apply to the taste of the pasta (multi-select 0=not apply, 1=apply):								Would you purchase this product if it were available at a reasonable price where you normally shop? (1=No; 2=Yes)	If there any comment that would you like to share to make further improvement? (Text)
				Umami	Bland	Soybean	Gummy	Moist	Chewy	Savoury	Roast		
1032	**5**	**402**	**5% Soy protein**	0	0	0	0	0	0	0	0	1	.
1033	**1**	**402**	**5% Soy protein**	0	0	1	0	0	1	0	0	1	No
1034	**4**	**402**	**5% Soy protein**	0	0	0	0	0	0	0	0	1	Taste like expired paste because sour flavour
1035	**2**	**402**	**5% Soy protein**	0	0	0	1	0	0	0	0	1	Better hardness needed.
1036	**5**	**402**	**5% Soy protein**	0	1	0	0	0	0	0	0	1	Too soft
1037	**4**	**402**	**5% Soy protein**	0	0	0	0	0	0	0	0	1	Need to reduce moisture
1040	**3**	**402**	**5% Soy protein**	0	0	0	0	1	1	0	0	1	Not acceptable
1001	**5**	**614**	**15% Soy protein**	0	1	0	0	1	0	0	1	1	No
1002	**4**	**614**	**15% Soy protein**	0	0	0	0	1	0	0	0	2	R
1003	**3**	**614**	**15% Soy protein**	0	0	0	0	1	0	0	0	1	Additional flavour requirements
1004	**2**	**614**	**15% Soy protein**	0	0	0	0	0	0	0	0	1	No comment
1005	**1**	**614**	**15% Soy protein**	0	1	0	1	0	1	0	0	1	Not good.
1006	**5**	**614**	**15% Soy protein**	0	1	0	0	0	1	0	0	1	Too sticky
1007	**4**	**614**	**15% Soy protein**	0	0	0	0	1	0	0	1	1	No
1008	**2**	**614**	**15% Soy protein**	0	0	0	0	0	0	0	0	1	Ry
1009	**1**	**614**	**15% Soy protein**	0	0	0	0	0	0	0	0	1	In my opinion, product 614 taste like incompletely cooked noodles, it has some raw flour texture. However, its flavour is good, and I like it.
1010	**3**	**614**	**15% Soy protein**	0	0	1	0	0	0	1	0	1	Bad taste
1011	**3**	**614**	**15% Soy protein**	0	0	0	0	0	0	0	0	1	.

Subject Code	Serving Position	Sample Identi-fier	Sample Name	Thick all that apply to the taste of the pasta (multi-select 0=not apply, 1=apply):								Would you purchase this product if it were available at a reasonable price where you normally shop? (1=No; 2=Yes)	If there any comment that would you like to share to make further improvement? (Text)
				Umami	Bland	Soybean	Gummy	Moist	Chewy	Savoury	Roast		
1012	**1**	**614**	**15% Soy protein**	0	1	0	1	0	0	0	0	1	Stickiness is high
1013	**5**	**614**	**15% Soy protein**	0	0	0	0	0	0	0	0	1	Not
1014	**4**	**614**	**15% Soy protein**	0	0	0	0	0	0	0	0	1	Bad after taste
1015	**2**	**614**	**15% Soy protein**	0	0	0	0	0	0	1	0	2	Somewhat tasty
1016	**2**	**614**	**15% Soy protein**	0	0	0	0	0	0	0	0	2	If colour is a bit lighter, that would be great
1017	**3**	**614**	**15% Soy protein**	0	1	0	0	0	1	0	0	1	Bland
1018	**1**	**614**	**15% Soy protein**	0	1	0	0	1	0	0	0	1	Quite bland
1019	**5**	**614**	**15% Soy protein**	0	0	0	0	0	0	0	0	1	I do not enjoy this one for its uncomfortable inside-month feeling
1020	**4**	**614**	**15% Soy protein**	0	0	0	0	0	0	0	0	2	Too soft but taste good
1021	**4**	**614**	**15% Soy protein**	0	0	0	1	0	1	0	0	1	Taste like sand in it
1022	**2**	**614**	**15% Soy protein**	0	1	0	0	0	0	0	0	1	Maybe change colour to light brown
1023	**3**	**614**	**15% Soy protein**	0	0	1	0	0	0	0	0	2	No thanks
1024	**5**	**614**	**15% Soy protein**	0	0	0	0	0	0	0	0	1	The texture is too dry, the appearance is not so attractive
1025	**1**	**614**	**15% Soy protein**	0	0	0	1	1	1	0	0	1	There is no aftertaste, which I like cause when you look at it, it does not have the most appealing appearance
1026	**4**	**614**	**15% Soy protein**	0	0	0	0	0	0	0	0	1	No

Subject Code	Serving Position	Sample Identi-fier	Sample Name	Thick all that apply to the taste of the pasta (multi-select 0=not apply, 1=apply):								Would you purchase this product if it were available at a reasonable price where you normally shop? (1=No; 2=Yes)	If there any comment that would you like to share to make further improvement? (Text)
				Umami	Bland	Soybean	Gummy	Moist	Chewy	Savoury	Roast		
1027	**3**	**614**	**15% Soy protein**	0	0	0	1	0	0	0	0	1	Very sticky to touch with a strong bitter, earthy aftertaste. It is also clumped together
1028	**5**	**614**	**15% Soy protein**	0	0	0	0	0	0	0	0	1	Do not like texture
1029	**2**	**614**	**15% Soy protein**	0	0	0	0	0	0	0	0	2	Colour a bit too dark
1030	**1**	**614**	**15% Soy protein**	0	0	1	0	0	0	0	0	1	It tastes a bit weird coz it is too soft and does not taste like pasta
1032	**4**	**614**	**15% Soy protein**	0	0	0	0	0	0	0	0	1	.
1033	**3**	**614**	**15% Soy protein**	0	0	0	0	0	0	0	0	1	No
1034	**2**	**614**	**15% Soy protein**	0	0	0	0	0	0	0	0	1	The ingredients taste wired
1035	**1**	**614**	**15% Soy protein**	0	0	0	0	0	0	0	0	1	More smooth appearance needed.
1036	**3**	**614**	**15% Soy protein**	0	0	1	0	0	1	0	0	1	Quite sticky
1037	**5**	**614**	**15% Soy protein**	0	0	0	0	0	1	0	0	1	Need to add interesting flavours
1040	**1**	**614**	**15% Soy protein**	0	1	0	0	0	1	0	0	1	I do not like it
1001	**3**	**875**	**5% Egg white**	0	1	0	0	1	0	0	1	2	Good
1002	**5**	**875**	**5% Egg white**	0	0	0	0	1	1	0	0	2	R
1003	**1**	**875**	**5% Egg white**	0	0	0	0	1	1	0	0	1	Little bit of additional flavour
1004	**4**	**875**	**5% Egg white**	0	1	0	0	0	0	0	0	1	No comment
1005	**2**	**875**	**5% Egg white**	0	1	0	1	0	0	0	0	2	Better than 614.
1006	**4**	**875**	**5% Egg white**	0	1	0	0	0	0	0	0	1	Too pasty
1007	**2**	**875**	**5% Egg white**	0	0	0	0	0	0	0	1	2	No

Subject Code	Serving Position	Sample Identi-fier	Sample Name	Thick all that apply to the taste of the pasta (multi-select 0=not apply, 1=apply):								Would you purchase this product if it were available at a reasonable price where you normally shop? (1=No; 2=Yes)	If there any comment that would you like to share to make further improvement? (Text)
				Umami	Bland	Soybean	Gummy	Moist	Chewy	Savoury	Roast		
1008	**1**	**875**	**5% Egg white**	0	1	0	1	0	1	0	1	2	Ok
1009	**3**	**875**	**5% Egg white**	0	0	0	0	0	0	0	0	2	This is an amazing experience for me. If it is hot enough that will be much better.
1010	**5**	**875**	**5% Egg white**	1	0	0	0	0	0	1	0	2	Too dark but homogeneous. Texture and flavour ok. The only big problem is the smell. Otherwise, it works.
1011	**1**	**875**	**5% Egg white**	0	0	0	0	1	0	0	0	1	..
1012	**2**	**875**	**5% Egg white**	0	1	0	0	0	1	0	0	2	Need improvement in the flavour
1013	**3**	**875**	**5% Egg white**	0	0	0	1	0	0	0	0	1	Not
1014	**5**	**875**	**5% Egg white**	0	0	0	0	0	0	0	0	1	Do not like the colour
1015	**4**	**875**	**5% Egg white**	0	0	1	0	1	1	1	0	2	No
1016	**1**	**875**	**5% Egg white**	0	0	0	1	0	0	0	0	1	Appearance should be improved
1017	**5**	**875**	**5% Egg white**	0	1	0	0	0	0	0	0	1	Bland
1018	**3**	**875**	**5% Egg white**	0	0	0	0	1	0	0	0	2	Flour like flavour
1019	**4**	**875**	**5% Egg white**	0	0	0	0	1	0	0	0	2	Very good for the difference of colour and flavour, which is not that strong as 137 and 402. And it tastes smooth as the 329
1020	**2**	**875**	**5% Egg white**	0	0	0	0	0	0	1	0	2	It is better

Subject Code	Serving Position	Sample Identi-fier	Sample Name	Thick all that apply to the taste of the pasta (multi-select 0=not apply, 1=apply):								Would you purchase this product if it were available at a reasonable price where you normally shop? (1=No; 2=Yes)	If there any comment that would you like to share to make further improvement? (Text)
				Umami	Bland	Soybean	Gummy	Moist	Chewy	Savoury	Roast		
1021	**5**	**875**	**5% Egg white**	0	0	0	1	0	1	0	0	1	Taste like sand in it and has strong smell
1022	**4**	**875**	**5% Egg white**	0	1	0	0	0	1	0	0	1	The pasta has grainy texture and colour and aroma should be improved
1023	**1**	**875**	**5% Egg white**	0	0	0	0	1	0	0	0	1	No thanks
1024	**3**	**875**	**5% Egg white**	0	0	0	0	0	0	0	0	1	The bitterness and texture (too soft)
1025	**2**	**875**	**5% Egg white**	0	1	1	1	0	1	0	0	2	It is gross
1026	**2**	**875**	**5% Egg white**	0	0	0	0	0	1	0	0	2	No
1027	**5**	**875**	**5% Egg white**	0	0	0	1	1	1	0	0	1	Has earthy after taste, and a bit sticky
1028	**4**	**875**	**5% Egg white**	1	0	0	0	0	0	0	0	1	Strong flavour
1029	**1**	**875**	**5% Egg white**	0	0	0	1	0	1	0	0	2	Taste a bit too strong
1030	**3**	**875**	**5% Egg white**	0	0	0	0	0	0	0	1	1	It tastes harder and can taste the fibre texture as well.
1032	**3**	**875**	**5% Egg white**	0	1	0	0	0	0	0	0	2	.
1033	**2**	**875**	**5% Egg white**	0	0	1	1	0	1	1	0	1	No
1034	**5**	**875**	**5% Egg white**	0	0	0	0	0	0	0	0	1	Do not like the texture too smooth
1035	**4**	**875**	**5% Egg white**	0	0	0	0	0	0	0	0	2	Better smooth appearance
1036	**4**	**875**	**5% Egg white**	0	1	1	0	0	0	0	0	2	Has an appreciable texture
1037	**2**	**875**	**5% Egg white**	0	0	0	1	0	1	0	0	1	Raw ingredients flavour
1040	**5**	**875**	**5% Egg white**	0	0	1	0	0	0	0	0	1	Not acceptable

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